ISOLATION AND IDENTIFICATION OF GALANGIN AND OTHER COMPOUNDS FROM *ALPINIA GALANGA* LINNAEUS WILLD AND *ALPINIA OFFICINARUM* HANCE

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

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การแยกและการพิสูจน์เอกลักษณ์ของกาแลนกินและสารอื่นจากข่าใหญ่ (*Alpinia galanga* Linnaeus Willd) และข่าเล็ก (*Alpinia officinarum* Hance)

นางสาวณัฐฐาพร สามารถ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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ณัฐฐาพร สามารถ : การแยกและการพิสูจน์เอกลักษณ์ของกาแลนกินและสารอื่นจากข่าใหญ่ (*ALPINIA GALANGA* LINNAEUS WILLD) และข่าเล็ก (*ALPINIA OFFICINARUM* HANCE). (ISOLATION AND IDENTIFICATION OF GALANGIN AND OTHER COMPOUNDS FROM *ALPINIA GALANGA* LINNAEUS WILLD AND *ALPINIA OFFICINARUM* HANCE) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สันติ ศักดารัตน์, 95 หน้า.

ข่าใหญ่และข่าเล็กเป็นพืชในวงศ์ Zingiberaceae จัดเป็นพืชสมุนไพรไทยที่รู้จักและนำมาใช้กันอย่างแพร่หลาย มีสรรพคุณต้านเชื้อแบคทีเรีย รักษาแผลพุพอง มีหนอง นอกจากนี้ยังมีคุณสมบัติเป็นยาฆ่าแมลงต่างๆ เมื่อนำส่วนสกัดหยาบจากเหง้าของข่าใหญ่มาทดสอบในเบื้องต้นเพื่อตรวจสารกาแลนกิน ซึ่งเป็นสารที่สนใจ ปรากฏว่าไม่พบแต่อย่างใด และเมื่อนำส่วนสกัดคลอโรฟอร์มของเหง้าจากข่าเล็กมาแยกด้วยเทคนิคทางโครมาโทกราฟี สามารถแยกสารบริสุทธิ์ได้ 3 ชนิด และพิสูจน์โครงสร้างของสารบริสุทธิ์ทั้งหมดที่แยกได้โดยใช้ข้อมูลทางสเปกโทรสโกปีจาก UV, IR, MS, ^{1}H-NMR และ ^{13}C-NMR ร่วมกับการเปรียบเทียบค่าที่ได้มีรายงานไว้แล้ว พบว่าสารที่ได้เป็นสารฟลาโวนอยด์ในกลุ่มฟลาโวนอล 2 ชนิด คือ 3,5,7-trihydroxyflavone (galangin) และ 3,5,7-trihydroxy-4′-methoxyflavone (kaempferide) รวมทั้งพบสารฟลาโวนอลในกลุ่มกลูโคไซด์ คือ 5,7-dihydroxy-4′-methoxy-3-*O*-β-D-glucopyranosideflavone (kaempferide-3-*O*-β-D-glucoside) ซึ่งสารนี้ยังไม่มีรายงานว่าพบในพืชชนิดนี้มาก่อน

สาขาวิชาเคมี ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา_____

NUTTAPORN SAMART : ISOLATION AND IDENTIFICATION OF GALANGIN AND OTHER COMPOUNDS FROM *ALPINIA GALANGA* LINNAEUS WILLD AND *ALPINIA OFFICINARUM* HANCE.

THESIS ADVISOR : ASST. PROF. SANTI SAKDARAT, Ph.D. 95 PP.

Alpinia Galanga Linnaues Willd and *Alpinia officinarum* Hance, a Thai medicinal plant of family Zingiberaceae, is well-known plant used in Thai traditional medicine as an antibacterial and antiulcer. Moreover it has insecticidal properties. The crude extract of *Alpinia Galanga* Linnaues Willd was preliminary tested and this test did not indicate any traces of the investigated compound (galangin). The chloroform crude extract of *Alpinia officinarum* Hance was purified by column chromatography and preparative thin-layer chromatography to give three pure compounds. Structural elucidation of the isolated compounds was carried out on the basis of spectral analyses, including UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR, as well as comparison with reported values. Two of these were identified as flavonol of flavonoids. They were 3,5,7-trihydroxy flavone (galangin) and 3,5,7-trihydroxy-4'-methoxy flavone (kaempferide). The other was 5,7-dihydroxy-4'-methoxy-3-*O*-*β*-D-glucopyranoside flavone (kaempferide-3-*O*-*β*-D-glucoside), which had not been previously reported in this species.

School of Chemistry Student's Signature_____

Academic Year 2007 Advisor's Signature_____

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I would like to thank my thesis advisor, Asst. Prof. Dr. Santi Sakdarat for his exceptional generous support, academic guidance and advice throughout the course of my graduate study at Suranaree University of Technology. My appreciation also goes to my committee members, Assoc. Prof. Dr. Malee Tangsathitkulchai, Asst. Prof. Dr. Thanaporn Manyum and Asst. Prof. Dr. Griangsak Eumkeb for their time and useful suggestions. I am grateful to Asst. Prof. Dr. Pichaya Trakanrungroj for her kindness and helpful comments. I would also like to thank all lecturers at the School of Chemistry, Suranaree University of Technology for their good attitude and advice.

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Finally, I dedicate this work to my parents and the rest of my family. They provided me every opportunity to succeed in my life and the encouragement I needed to succeed.

Nuttaporn Samart

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LIST OF ABBREVIATIONS

^{o}C	degree Celcius
$CDCl_3$	chloroform-d_1
cm	centimeter
cm^{-1}	wave number unit
d	doublet
dd	doublet of doublets
g	gram
g/kg	gram per kilogram
HPLC	high performance liquid chromatography
Hz	Hertz
IR	infrared spectroscopy
J	coupling constant in Hertz
m	multiplet
mg/mL	milligram per milliliter
mL	milliliter
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MS	mass spectrophotometry
nm	nanometer
NMR	nuclear magnetic resonance spectroscopy

LIST OF ABBREVIATIONS (Continued)

ppm	parts per million
PTLC	preparative thin-layer chromatography
q	quartet
s	singlet
t	triplet
TLC	thin-layer chromatography
UV	ultraviolet radiation
UV-Vis	ultraviolet-visible radiation
δ	chemical shift in ppm
λ_{max}	maximum absorption wavelength
μg	microgram
μg/mL	microgram per milliliter
ν_{max}	maximum absorption wavenumber

CHAPTER I

INTRODUCTION

1.1 General introduction

Study on natural products is always an interesting target for scientists over decades, especially on plants. Historically, plants (fruits, vegetables, medicinal herbs, etc.) have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen containing compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities, e.g., antioxidant, anti-inflammatory, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, or antiviral activities. In many oriental countries (China, Thailand, Japan, etc.), the traditional herbal medicines have been widely used for thousands of years. Herbal plants have become the main object of chemists, biochemists, and pharmacists. Their research plays an important role for discovering and developing new drugs, which hopefully are more effective than and have no side actions like most modern drugs (Ly, Yamauchi, Shimoyamada, and Kato, 2002; Juntachote, Berghofer, Siebenhandl, and Bauer, 2006).

Marketed plants are very important items of trade in many parts of the world. The community uses these for a variety of purposes such as food, cosmetics, flavors, spices, and medicines. It seems that plants used for medicinal purposes form the most common category (Juntachote *et al*., 2006).

Galangal (also called galanga, galingale, or galangale) is a species of the ginger family (Zingiberaceae) which is composed of 47 genera and 1400 species distributed world wide (Pothisiri, 1996). Three species of galangal, *Alpinia officinarum* Hance, *Alpinia galanga* Linnaeus Willd and *Alpinia conchigera* Griff, are commonly known and found in Thailand. Their traditional uses are shown in Table 1.1.

Table 1.1 Thai medicinal plants of the family Zingiberaceae and their traditional uses (Athamaprasangsa *et al*., 1994; Mallavarapu, 2002; Kanjanapothi *et al*., 2004).

Species of *Zingiberaceae* Family	**Traditional Uses**
***Alpinia officinarum* Hance**	Rhizomes: healing ring worm, venereal diseases, carminative, abdominal discomfort, stomach and discomfort Leaves: healing ring worm Tree: healing smallpox
***Alpinia galanga* (L.)Willd**	Rhizomes: healing contusion, stomach discomfort, abdominal discomfort, and squeamishness, expectorant, blood tonic Leaves and Flowers: healing ring worm

Table 1.1 (Continued)

Species of *Zingiberaceae* Family	**Traditional Uses**
***Alpinia conchigera* Griff**	Rhizomes: expectorant, healing venereal diseases, tympanites, and carminative

Three species of galangal, kaempferia galangal (*Alpinia conchigera* Griff), smaller galangal (*Alpinia officinarum* Hance), and greater galangal (*Alpinia galanga* (L.) Willd), are used in Thai food cooking and some are known to have medical properties. Galangal has characteristic fragrance and mildly spicy taste. Similar to other species of the ginger family, galangal warms and comforts digestion. It can be used in all conditions where the central areas of the body need greater warmth making it ideal as a treatment for pain due to chest cold. This herb is mostly used together with nutgrass, flatsedge and rhizome (*Rhizoma Cyperi*) in order to soothe the liver, dispel the cold and to relieve pain. It was found that eating five daily servings of its vegetables and fruits decreased the chances of cancer, and eating nine or ten daily servings, together with three servings of low-fat dairy products lowered blood pressure. In traditional Chinese herbal medicine, galangal is a warming herb used for abdominal pain, vomiting, and hiccups, as well as diarrhea due to internal symptoms attributed to cold. When used for hicupps, galangal is combined with codonopsis as an effective treatment. In India and in Southwestern Asia, galangal is considered stomachic, anti-inflammatory, expectorant and a nervine tonic. Galangal is used in the treatment of hiccups, dyspepsia, stomach pain, rheumatoid arthritis, and intermittent fever. Galangal was brought to Europe by Arabian physicians more than a thousand

years ago. Galangal is used in western countries for gas, indigestion, vomiting, and stomach pain. An infusion can be used to alleviate painful aphthous ulcer (aka canker sores) and painful gums. Galangal has also long been recommended as a treatment for seasickness (Lysias-Derrida, www, 2006).

Rhizomes of galangal have three groups of important chemical constituents, flavonoids, glycosides, and diarylheptanoids. It has been reported that galangal has biological activities, including antitumor, antiulcer, antibacterial, and antifungal properties (Itokawa, Morita, Midorikawa, Aiyama, and Morita, 1985; Newman, Cragg, and Snader, 2003; Matsuda, Ando, Kato, Morikawa, and Yoshikawa, 2006).

Flavonoids are compounds which all possess the same C_{15} (C_6-C_3-C_6) flavone nucleus (Harborne, 1988; Macheix, Fleuriet, and Jay-Allemand, 1990): two benzene rings (A and B) linked through an oxygen containing pyran or pyrone ring (C). This structure is common to 3-deoxyflavonoids (flavones, flavanones, and isoflavones) and 3-hydroxyflavonoids (anthocyanin, flavan-3-ol, flavan-3,4-diols, flavanonols, and flavonols) (Khnau, 1976; Middleton and Kandaswami, 1993) as shown in Table 1.2.

Table 1.2 The chemical structures of the flavonoids family

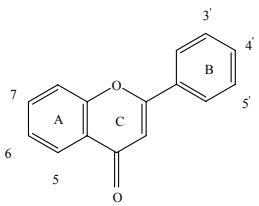
Structural formula	**Representative flavonoids**	**Substitutions at position**					
		5	**6**	**7**	**3′**	**4′**	**5′**
Flavone	Apigenin	OH	H	OH	H	OH	H
	Chrysin	OH	H	OH	H	H	H
	Tectochrysin	OH	H	OH	H	H	H
	Luteolin	OH	H	OH	OH	OH	H

Table 1.2 (Continued)

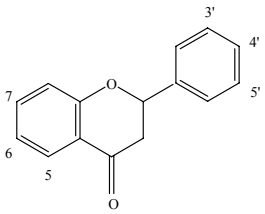
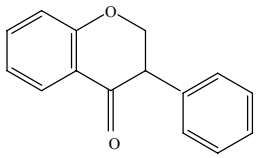
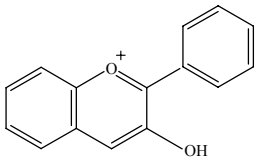
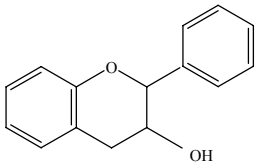
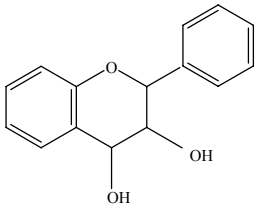
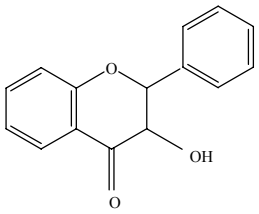
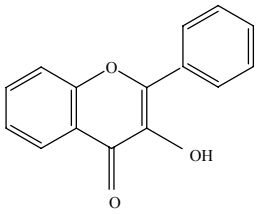
Structural formula	Representative flavonoids	Substitutions at position					
		5	**6**	**7**	**3′**	**4′**	**5′**
Flavanone							
	Eriodictyol	OH	H	OH	OH	OH	H
	Naringenin	OH	H	OH	H	OH	H
Isoflavone							
	Daidzein	H	H	OH	H	OH	H
	Genistein	OH	H	OH	H	OH	H
Anthocyanin							
	Aurantinidin	OH	OH	OH	H	OH	H
	Cyanidin	OH	H	OH	OH	OH	H
Flavan-3-ol							
	Flavanolok	H	H	H	H	H	H
Flavan-3,4-diols							
	Flavandiolok	H	H	H	H	H	H

Table 1.2 (Continued)

Structural formula	Representative flavonoids	Substitutions at position					
		5	**6**	**7**	**3′**	**4′**	**5′**
Flavanonol							
	Pinobanksin	OH	H	OH	H	H	H
	Taxifolin	OH	H	OH	OH	OH	H
Flavonol	Kaempferol	OH	H	OH	H	OH	H
	Myricetin	OH	H	OH	OH	OH	OH
	Quercetin	OH	H	OH	OH	OH	H
	Galangin	OH	H	OH	H	H	H

Galangin is a member of the flavonol class of flavonoids and is present in high concentration in honey and *Alpinia officinarum* Hance. Galangin was first isolated in 1881 from the rhizomes of galangal, *Alpinia officinarum* Hance (Wollenweber, 1988) and synthesized by Heap and Robinson (1926). Since then, it has been found in extracts of many plants (Ferraro, Norbedo, and Coussio, 1981; Wollenweber and Valant-Vetschera, 1985; McDowell, Lwande, Deans, and Waterman, 1988). Galangin, chrysin, tectochrysin, pinobanksin, and pinocembrin are characteristic flavonoids from propolis (bee glue) and bee wax (Thomas-Barberan, Ferreres, Tomas-Lorente, and Ortis, 1993; Ferreres, Andrade, and Thomas-Barberan, 1994; Johnson, Eischen, and Giannasi, 1994; Soler, Gil, Garcia-Viguera, and Thomas-Barberan, 1995). It is a major constituent of fruits and vegetables (Phang, Poore,

Lopaczynska, and Yeh, 1993). It was, however, implicated together with quercetin and kaempferol in the increase in the frequency of chromosomal aberrations and mutations in Chinese hamster ovary cells (Middleton and Kandaswami, 1993). Information on the antimicrobial property of galangin is scanty. Nishino, Nobuyasu, Shinkichi, Akihisa, Koji, and Masako (1978) reported galangin as the most active compound of the 26 flavonoids tested on *Staphylococcus epidermidis*. Chabot, Bel-Rhlid, and Piche (1992) observed a reduction in the hyphal growth of *Gigaspora margarita* by galangin. Kanazawa, Kawasaki, Samejima, Ashida, and Danno (1995) reported the demutagenic activity of galangin and quercetin on *Salmonella typhimurium*.

1.2 Specification of galangal

In this study, the extraction, purification and identification of galangin and other isolated compounds from the rhizomes of greater galangal and smaller galangal are investigated.

1.2.1 Specification of greater galangal

Botanical name: *Alpinia galanga* Linnaeus Willd

Common names: ข่าใหญ่, ข่าหลวง (Kha-luang), false galangal, galangal, greater galangal, grand galangal, galanga majeur, grober galgant, stor kalanga of *Alpinia galanga* (L.) Willd, etc (เต็ม สมิตินันทน์. 2524 and นันทวัน บุญประภัศร. 2541).

Botanical description**:** It is an annual crop herb, between one and two meter in height, depending on variety. The leaves are 25-35 cm long, and rather narrow

blades. Inflorescence is a terminal raceme on a bladeless leaf-sheath. The flowers are borned at the top of the plant and are small, white and streaked with deep-red veining.

The rhizome resembles ginger in shape, but it is much higher. Some varieties have a yellow skin and the interior is nearly white. The rhizomes are tough and difficult to break. It prefers rich and moist soil in a protected and shady position and is tender in drought and frost. Fruits are ellipsoid and light brown which become dark brown when mature. (Grieve, www, 1995-2008). The morphological features of greater galangal parts are illustrated in Figure 1.1 and 1.2.

Figure1.1 Morphological features of rhizome, leave, and flower of greater galangal (Grieve, www, 1995-2008)

Figure 1.2 Pictures of greater galangal appearing in nature

Ecology and Distribution**:** The plant springs after all danger of frost is past and the soil has warmed up at a depth of 5-10 cm. The plants require a rather hot and moist climate, and well-drained, humous or loamy soil. They are commonly cultivated throughout nature.

Propagation: They are propagated by division of the rhizomes.

Parts used: The rhizome is used for indigestion, colic, dysentery and cancer of the stomach. It is a remedy for food poisoning. The grated rhizome is prescribed for spleen trouble and herpes, an infusion of the leaves is for stimulant and antirheumatism. The fresh rhizome is also used as a spice for flavoring food.

1.2.2 Specification of smaller galangal

Botanical name: *Alpinia officinarum* Hance

Common names: ข่าเล็ก, galangal, smaller galangal, galangal minor, lesser galangal, Siamese ginger, Chinese ginger, China root, India root, aromatic ginger,

kaempferia galanga, rhizoma galanga of *Alpinia officinarum* Hance, etc (เต็ม สมิติ-นันทน์. 2524 and นันทวัน บุญประภัศร. 2541).

Botanical description**:** The size is smaller than the greater galangal as the name implies. The leaves are long and slender, roughly half of the dimensions of the greater galangal. The herb grows to a height of about 5 feet. The leaves are long and rather narrow blades. The flowers are borned in terminal spike. The petals are white, with deep-red veining distinguishing the lippetal. The branched pieces of rhizome are from 1.5 to 3 inches in length, and seldom more than 0.75 inch thick. Fruits are globose and orange-red which become black when mature (Grieve, www, 1995-2008). The morphological features of smaller galangal parts are shown in Figure 1.3 and 1.4.

Ecology and Distribution**:** The plants require a rather moist climate, and well-drained, humous or loamy soil. They are commonly cultivated throughout tropical rainforest.

Propagation: They are propagated by division of the rhizomes.

Parts used: Rhizomes are used as antiflatulence and in topical treatment for ring worm.

1.3 Bioactivities of galangin

Antimicrobial properties of medicinal plants have been reported from different parts of the world including Thailand, Malaysia, and China in which indigenous cultures possess a rich heritage of healing with medicinal plants (Ahmad and Beg, 2001; Mehmood and Mohammad, 1998; Brantner and Grein, 1994).

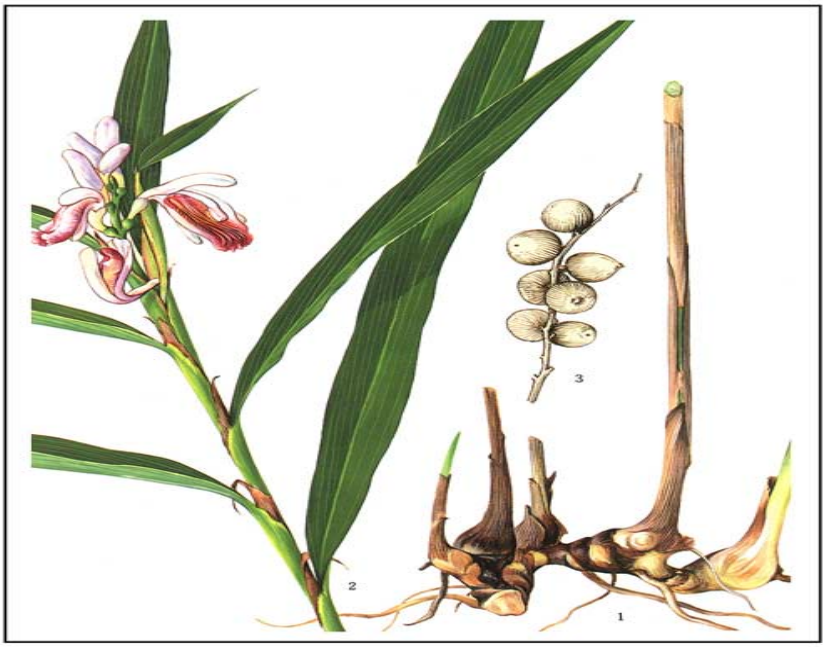


Figure1.3 Morphological features of rhizome, leave, fruits, and flower of smaller galangal (Grieve, www, 1995-2008)

Figure 1.4 Pictures of smaller galangal appearing in nature

The comparison of the rhizomes of both galangals are shown in Figure 1.5.

(a) (b)

Figure 1.5 Rhizomes of (a) greater galangal and (b) smaller galangal

1.3.1 Effect against herpes simplex virus (HSV)

Amoros, Simoes, Girre, Sauvager, and Cormier (1992) reported a comparative assay of the antiviral activity of galangin, kaempferol and quercetin on HSV-1 in Vero cells. Galangin was reported to be the most active, being able to reduce the viral titer by 10.8 μg/mL. Based on 50 percent inhibited dose, the results showed that galangin inhibited plaque formation by HSV-1.

Bacon, Jones, McGrath, and Johnston (1996) reported similar activities in the plaque reduction assay with 50 percent effective concentrations of 0.8 and 0.6 μg/mL of galangin, respectively.

1.3.2 Effect against the carcinogenic potential of overcooked, char-grilled foods

Several studies showed that galangin may have a potent anti-cancer effect, especially through inhibition of the detoxification enzyme CYP1A1 and modulation of the aryl hydrocarbon receptor. This implies that galangin has a protective effect against the carcinogenic potential of overcooked, char-grilled foods. Galangin is also a potent preserver of glutathione, an endogenous free radical scavenger (Lysias-Derrida, www, 2006).

1.3.3 Effect on inhibition of enzymes in cyclo-oxygenase family

Galangin exhibits a strong inhibitory effect on the cyclo-oxygenase family of enzymes. This provides a strong cardioprotective effect by inhibiting the aggregation of platelets, and providing a consistent systemic anti-inflammatory effect (Lysias-Derrida, www, 2006).

1.3.4 Effect on prevention of heart disease and lipid peroxidation

Galangin can prevent heart disease and has anti-oxidative effect on endothelial tissues. Therefore, it helps preserve other protective antioxidants such as vitamin E, vitamin C, and other flavonoids, and also can prevent lipid peroxidation (Lysias-Derrida, www, 2006).

1.4 Biosynthesis of flavonols

A key step of flavonoid biosynthesis is the condensation of three molecules of malonyl-CoA with *p*-coumaroyl-CoA to form the C_{15} intermediate 4,2',4',6'-tetrahydroxychalcone (Harborne, 1988; Strack, 1997) as shown in Figure 1.6. The enzyme catalyzing this step is chalcone synthase. For all chalcone synthases tested so

far, *p*-coumaroyl-CoA is the best substrate. Normally, it appears that the second B-ring hydroxyl is inserted at a later stage to give flavonoids with 3',4'-di-OH substitution (Harborne, 1988; Maria, Anna, Lorenzo, Giovanni, and Franco, 2007).

The next step after chalcone synthesis is its stereospecific isomerization to a (2*S*)-flavanone, ((2*S*)naringenin), catalyzed by chalcone isomerase. Flavanones represent a branch point in the biosynthesis since they may be converted to either flavones (e.g. apigenin) or to isoflavones (e.g. genistein). The next enzyme along the pathway, flavanone-3-hydroxylase catalyzes the conversion of (2*S*)-naringenin to (2*R*,3*R*)-dihydrokaempferol and also (2*S*)-eriodictyol to (2*R*,3*R*)-dihydroquercetin (Britch and Grisebach, 1986). The enzyme flavonol synthase converts dihydrokaempferol to kaempferol (Spribille and Forkmann, 1984). The enzymatic hydroxylation of the flavonol ring B at C-3' and C-5' has been demonstrated although the possibility that, in some cases, *p*-coumaric acid is hydroxylated to caffeic acid before being incorporated into the flavonoid molecule, has not been ruled out. Additional hydroxylations can apparently occur at virtually all levels of oxidation of the flavonoid skeleton (Britton, 1983).

Most of the flavonoids occur as glycosides in actively metabolizing plant tissues. There are hundreds of different glycosides, with glucose, galactose, rhamnose, xylose and arabinose as the most frequently found sugar moieties (Strack, 1997). The two major types of linkages are O- and C-glycosides (Harborne, 1994). Glycosyl transferase catalyzes the glycosylation of flavonoids. Flavonoid conjugation is not restricted to glycosylation. Many flavonoids contain acylated sugars. The acyl groups are either hydroxycinnamates or aliphatic acids such as malonate (Strack, 1997). In

the acylation reaction, the sugar hydroxyl and acid groups undergo esterification reaction (Markham, 1989).

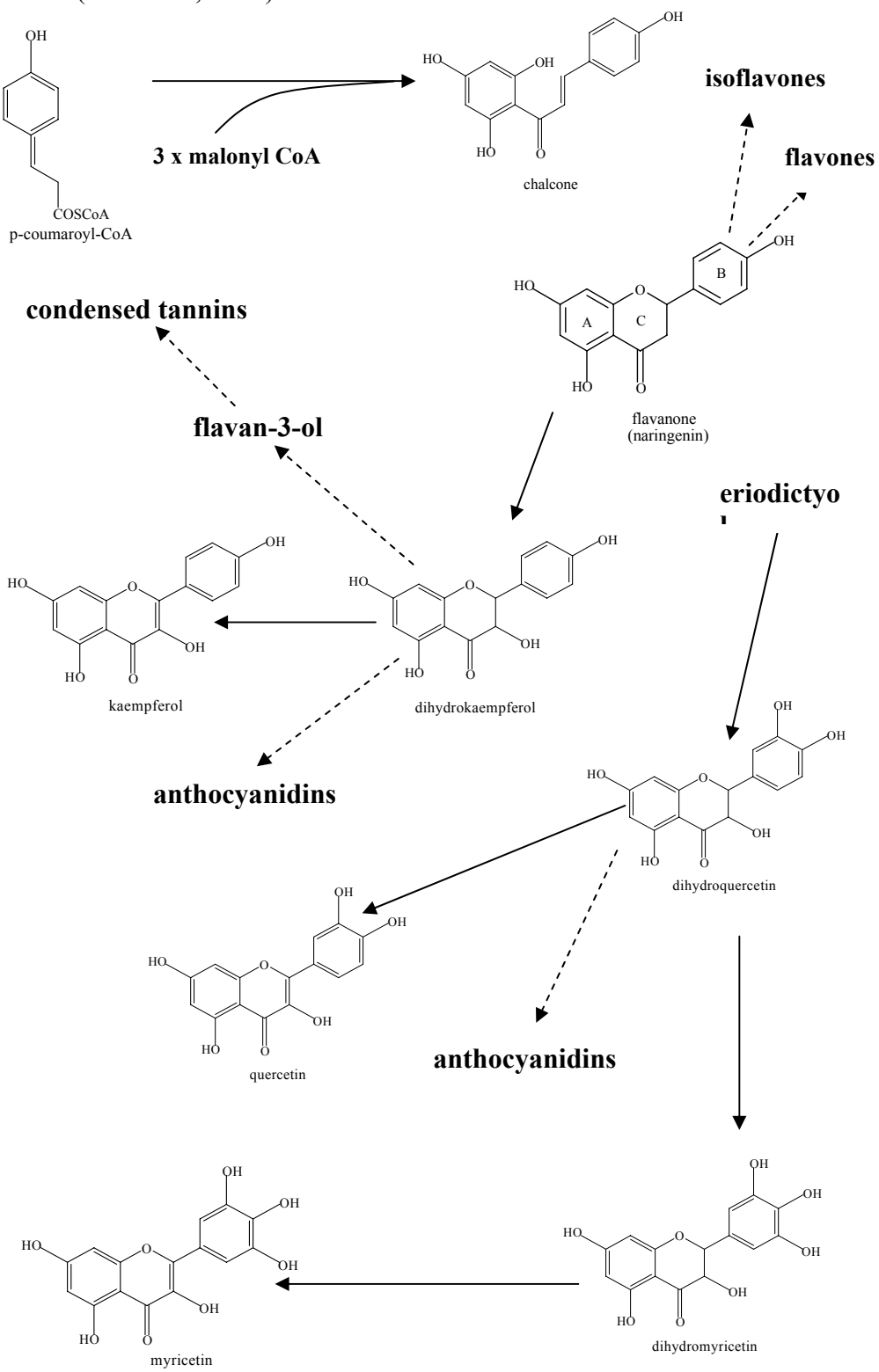


Figure 1.6 Biosynthesis of flavonoids (Strack, 1997)

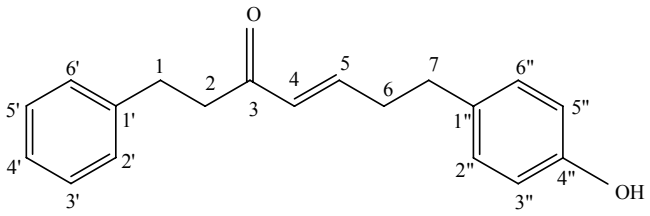
1.5 Importance of galangin

At present, bacterial resistance to β-lactam antibiotics is a global problem. Today over 90% of *Staphylococus aureus* (*S. aureus*) strains are β-lactamase positive. Strains of β-lactam-resistant *S. aureus* including methicillin-resistant *S. aureus* (MRSA) now pose serious problem to hospitalized patients, and their care providers (Mulligan, 1993). Antibiotics available for the treatment of MRSA infection are fairly toxic and their use is frequently associated with unwanted side-effects (Brumfitt and Hamilton-Miller, 1989). Novel antibiotics and/or new approaches that can reverse the resistance towel tried agents which have lost their original effectiveness are research objectives of far reaching importance (Reading and Cole, 1977). The basis research reported that the isolated of galangin from *Zingiberaceae* family (*Alpinia officinarum* Hance, *Alpinia nigra* (Gaertn) Burtt, and *Alpinia galanga* (L.) Willd) has inhibitory effect, against β-lactam-resistant *S. aureus* and MRSA when used alone and in combination with β-lactam antibiotics group (Teubert, Wunscher, and Herrmann, 1977). Eumkeb and Richards (2003) as well as Oonmetta-aree, Suzuki, Gasaluck, and Eumkeb (2006) reported that galangin exhibited the potential to reverse bacterial resistance to β-lactam antibiotics against MRSA and other strains of β-lactam-resistant *S. aureus*.

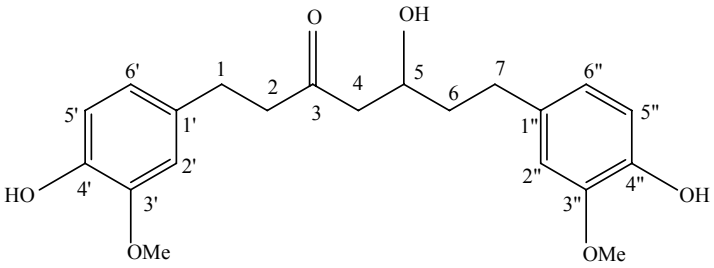
1.6 Chemical constituents of galangal

Itokawa *et al.* (1985) reported the isolation of six diarylheptanoids, including 7-(4″-hydroxyphenyl)-1-phenyl-4-hepten-3-one **(1)**, 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-3-heptanone (5-epihexahydrocurcumin) **(2)**, 5-methoxy-7-(4″-

hydroxyphenyl)-1-phenyl-3-heptanone **(3)**, 5-methoxy-1,7-diphenyl-3-heptanone **(4)**, 5-hydroxy-1,7-diphenyl-3-heptanone (5-epidihydroyashabushiketol) **(5)**, and 5-hydroxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptanone **(6)** from chloroform extract of the rhizomes of smaller galangal.



(1)



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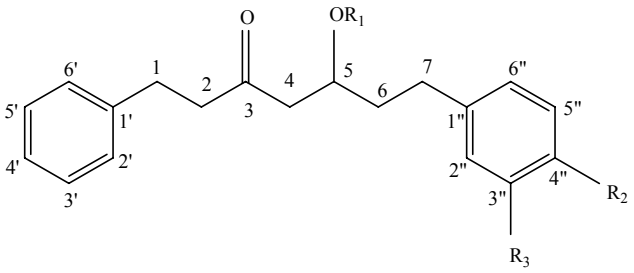


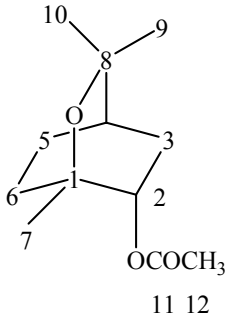
(3) : R_1 = Me, R_2 = OH, R_3 = H

(4) : R_1 = Me, R_2 = H, R_3 = H

(5) : R_1 = H, R_2 = H, R_3 = H

(6) : R_1 = H, R_2 = OH, R_3 = OMe

Kikue, Nakamura, and Kobayashi (1998) isolated four isomers of acetoxycineoles, *trans*-2-acetoxy-1,8-cineole **(7)**, *trans*-3-acetoxy-1,8-cineole **(8)**, *cis*-3-acetoxy-1,8-cineole **(9)**, and *cis*-2-acetoxy-1,8-cineole **(10)** from diethyl ether extract of the rhizomes of greater galangal.



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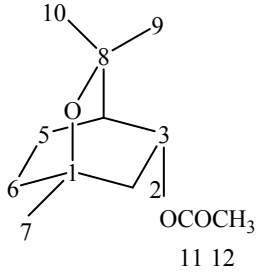

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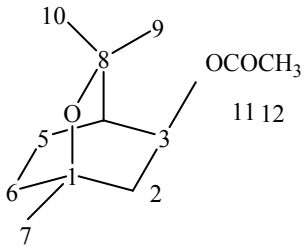

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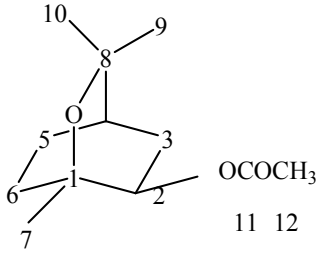
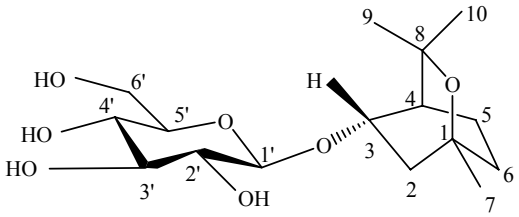


(10)

Ly *et al*. (2002) reported the isolation of nine glucosides, (1*R*,3*S*,4*S*)-*trans*-3-hydroxy-1,8-cineole *β*-D-glucopyranoside **(11)**, 3-methyl-2-buten-1-yl *β*-D-glucopyranoside **(12)**, benzyl *β*-D-glucopyranoside **(13)**, 1-*O*-*β*-D-glucopyranosyl-4-allylbenzene (chavicol *β*-D-glucopyranoside) **(14)**, 1-hydroxy-2-*O*-*β*-D-glucopyranosyl-4-allylbenzene **(15)**, 1-*O*-*β*-D-glucopyranosyl-2-hydroxy-4-allylbenzene (demethyleugenol *β*-D-glucopyranoside) **(16)**, 1-*O*-(6-*O*-*α*-L-rhamnopyranosyl-*β*-D-glucopyranosyl)-2-hydroxy-4-allylbenzene (demethyleugenol *β*-rutinoside) **(17)**, 1-*O*-(6-*O*-*α*-L-rhamnopyranosyl-*β*-*D*-glucopyranosyl)-4-allylbenzene (chavicol *β*-ruti-

noside) **(18)**, and 1,2-di-*O*-*β*-D-glucopyranosyl-4-allylbenzene **(19)** from methanol extract of the fresh rhizomes of smaller galangal.



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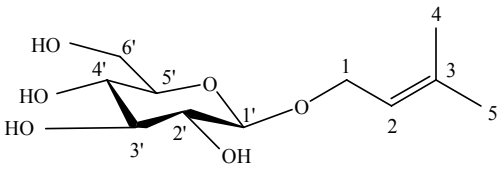

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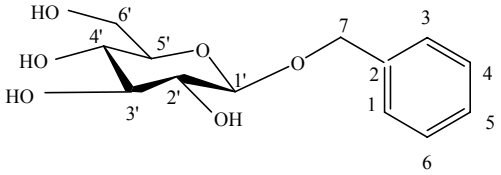

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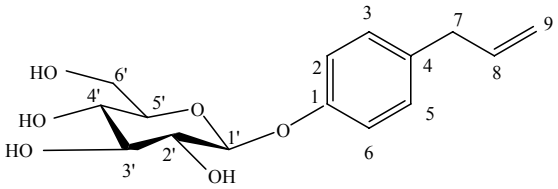

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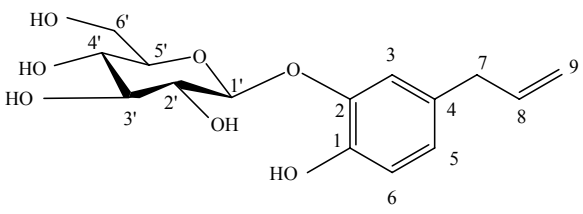

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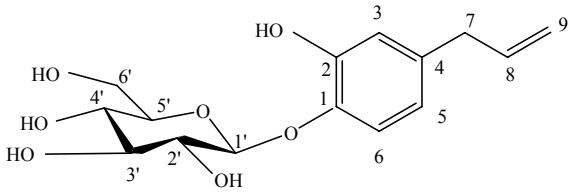

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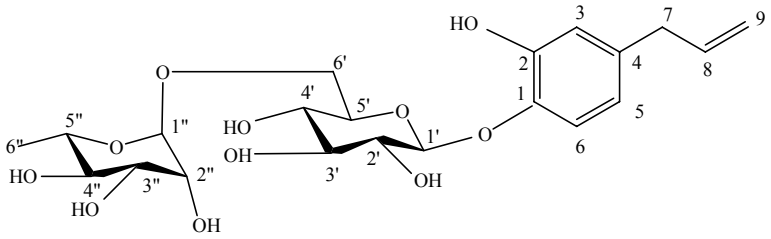

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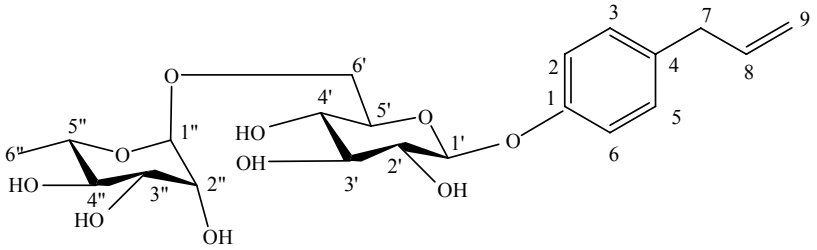

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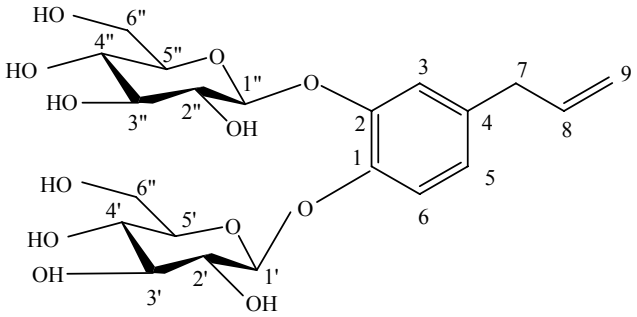
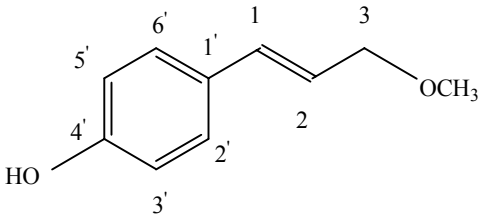


(19)

Ly, Shimoyamada, Kato, and Yamauchi (2003) reported the isolation of seven phenylpropanoids, (*E*)-*p*-coumaryl alcohol γ-*O*-methyl ether **(20)**, (*E*)-*p*-coumaryl alcohol **(21)**, (4*E*)-1,5-bis(4-hydroxyphenyl)-1-methoxy-2-(methoxymethyl)-4-pentene **(22)**, (4*E*)-1,5-bis(4-hydroxyphenyl)-1-ethoxy-2-(methoxymethyl)-4-pentene **(23)**, (4*E*)-1,5-bis(4-hydroxyphenyl)-1-[(2*E*)-3-(4-acetoxyphenyl)-2-propenoxy]-2-(methoxymethyl)-4-pentene **(24)**, (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(methoxymethyl)-4-penten-1-ol **(25)**, (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol **(26)** from methanol extract of the fresh rhizomes of smaller galangal.



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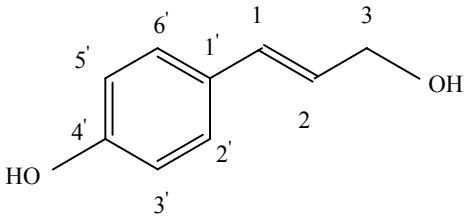

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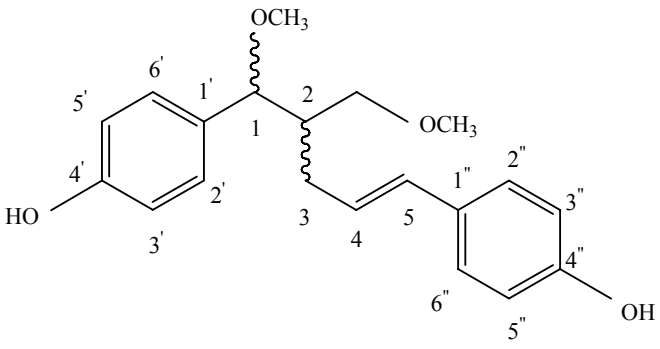

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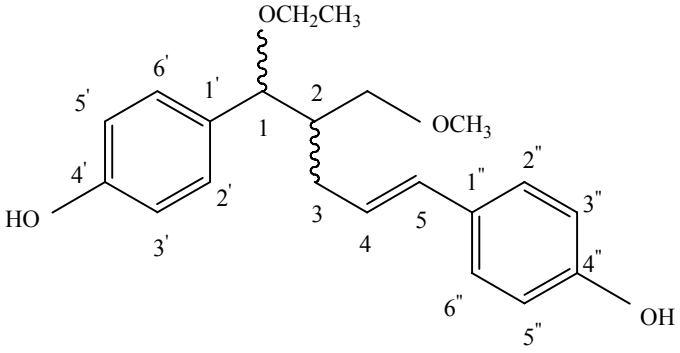

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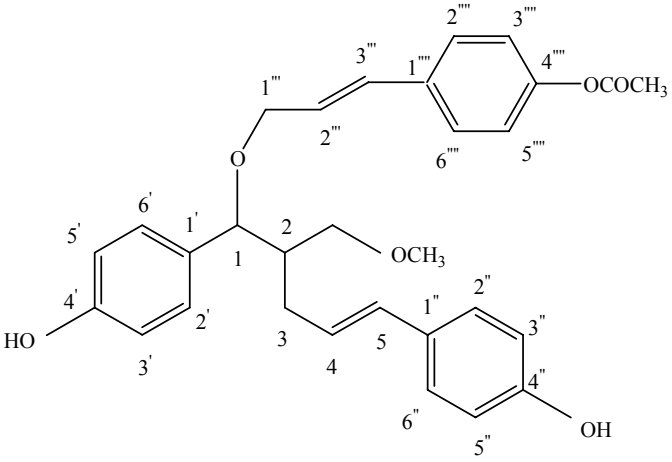

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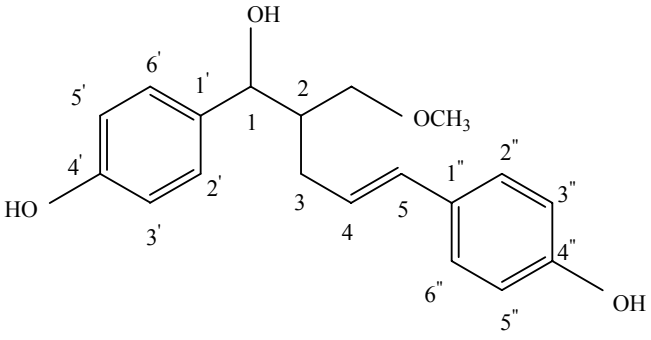

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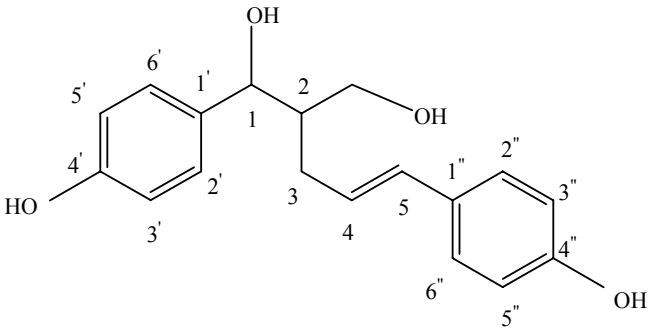
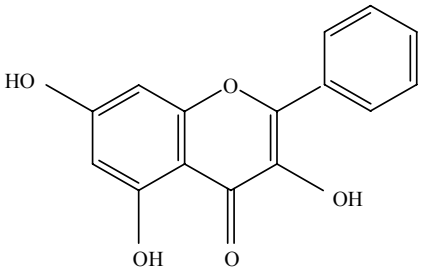


(26)

Hisashi, Shin, Tomoko, Toshio, and Masayaki (2005) isolated three flavonoids, galangin **(27)**, kaempferide **(28)**, and pinobanksin **(29)**, 3-phenylpropanoic acid **(30),** and zingerone **(31)** from ethyl acetate and water soluble portions of the ethyl acetate extract of the rhizomes of smaller galangal.



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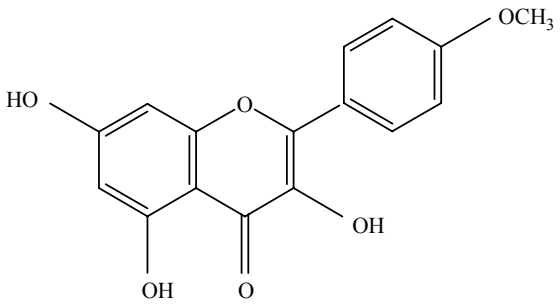

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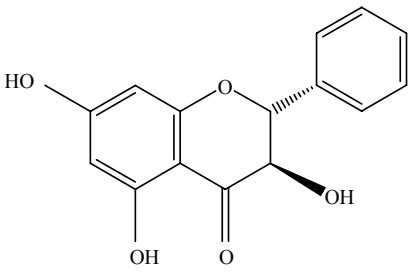

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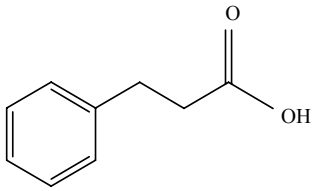

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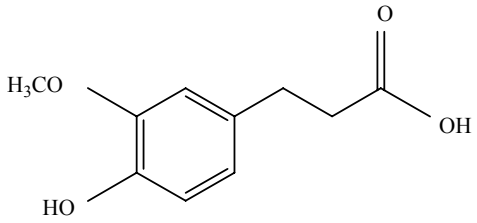
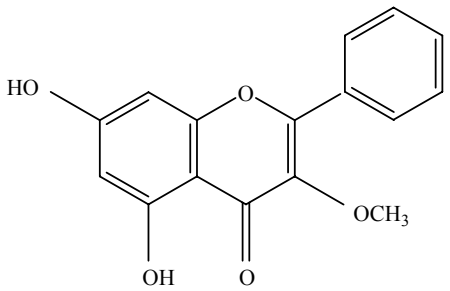


(31)

Tao, Wang, Zhu, Lu, and Wei (2006) isolated two flavonoids, galangin **(27)**, and 3-*O*-methyl galangin **(32)** from methanol extract of the rhizomes of smaller galangal.



(32)

Our research group has been interested in galangin due to its potential to reverse bacterial resistance to *β*-lactam antibiotics against MRSA and other strains of *β*-lactam-resistant *S. aureus* to be studied by Eumkeb's group.

The objectives of this research were as follows:

1. To extract and isolate galangin and other compounds from the rhizomes of greater galangal and smaller galangal.

2. To identify the structures of the isolated substances.

CHAPTER II

MEDICINAL PLANT MATERIALS AND OVERVIEW OF THE ANALYTICAL METHODS

2.1 Factors affecting efficacy and safety of medicinal plant materials

There are some important factors affecting the efficacy and safety of medicinal plant materials, namely: (Dechatiwongse na Ayudhaya, 1997; Chavalittumrong *et al*., 2001).

2.1.1 Plant name

There are some difficulties with the plant names because some plants have the same local name but belong to different botanical origins or vice versa. If wrong medicinal plant materials are used, they will possess not only undesirable activities, but also may have unexpected toxicities.

2.1.2 Part used

The right part of a plant should be used. Different parts generally contain different substances.

2.1.3 Age

The proper harvesting time for medicinal plants should be known to obtain high content of therapeutic principles.

2.1.4 Preparation and storage

The suitable processes of cleaning, cutting, drying, and preservation should be followed to prevent the contamination or deterioration of crude plant materials.

2.1.5 Quality

Though we know the correct plant name, the right part used, the proper harvesting time, suitable process of preparation and storage, we still cannot assume that raw materials of high quality can be obtained. Chemical constituents of plants are usually affected by external factors; e.g. climate, the physical features of the land, irrigation, fertilizer, etc.

Hence, it is essential to set up scientific criteria for standardization and quality control of herbal medicines to ensure that the consumers receive drugs of high quality.

2.2 Standard specifications of medicinal plant materials

The standard control of herbal remedies is performed by 2 aspects.

2.2.1 Establishment of standard specifications of herbal raw materials according to World Health Organization (WHO) documents and other useful information

The specifications for medicinal plant materials should include the following (Dechatiwongse na Ayudhaya, 1997; Chavalittumrong, 2000 and 2002; Chavalittumrong *et al*., 2001):

1. Name (scientific name, English name, local name)
2. Synonyms
3. Definition
4. Constituents
5. Plant description
6. Description of plant material

6.1 General appearance

6.2 Organoleptic properties

6.3 Microscopic characteristics

7. Packaging and storage

8. Identification

8.1 Chemical reaction

8.2 Thin-layer chromatographic analysis

9. Purity tests

9.1 Microbiological

9.2 Foreign matter

9.3 Total ash

9.4 Acid-insoluble ash

9.5 Water-soluble extract

9.6 Alcohol-soluble extract

9.7 Moisture

9.8 Pesticide residues

9.9 Arsenic and heavy metals

9.10 Radioactive residues

10. Determination of major/active chemical constituents

11. Medical uses and doses

2.2.2 Development of good manufacturing practice (GMP) guidelines for the manufacture of herbal medicinal products

The purpose of these guidelines is to outline steps which should be taken, as necessary and appropriate, by manufacturers of herbal medicinal products with the

objective of ensuring that their products are of the nature and quality intended. Successful and concurrent implementation of these GMP guidelines will not only improve the image of the herbal medicinal products industry, but also promote international recognition and acceptance, and consequently, escalate the export potential of these products. For the good manufacturing practices for pharmaceutical products, supplementary guidelines for the manufacture of herbal medicinal products; (WHO/Pharm/93.56) should be followed (Lou, 1993; Dechatiwongse na Ayudhaya, 1997; Chavalittumrong, 2000 and 2002; Chavalittumrong *et al*., 2001).

Nowadays, active constituents of drugs are important. An active constituent is truly responsible for therapeutic activity of a medicinal plant. The extracts are further subjected to chemical tests for identification of the plant constituents. The isolated constituents are of further importance to the pharmaceutical industry for applied research (Tao *et al*., 2006).

2.3 An overview of the analytical methods

2.3.1 Extraction theory (soxhlet extraction)

Soxhlet extraction is convenient and widely used for extraction because of its continuous process. The powdered plant is placed in a soxhlet apparatus, which is on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to flask and the setup is heated under reflux. The steam of solvent, which contacts with the material, will dissolve the metabolites and bring them back to the flask. A soxhlet extractor is illustrated in Figure 2.1 (Wingrove, Thompson, Wafford, and Whiting, 1981).

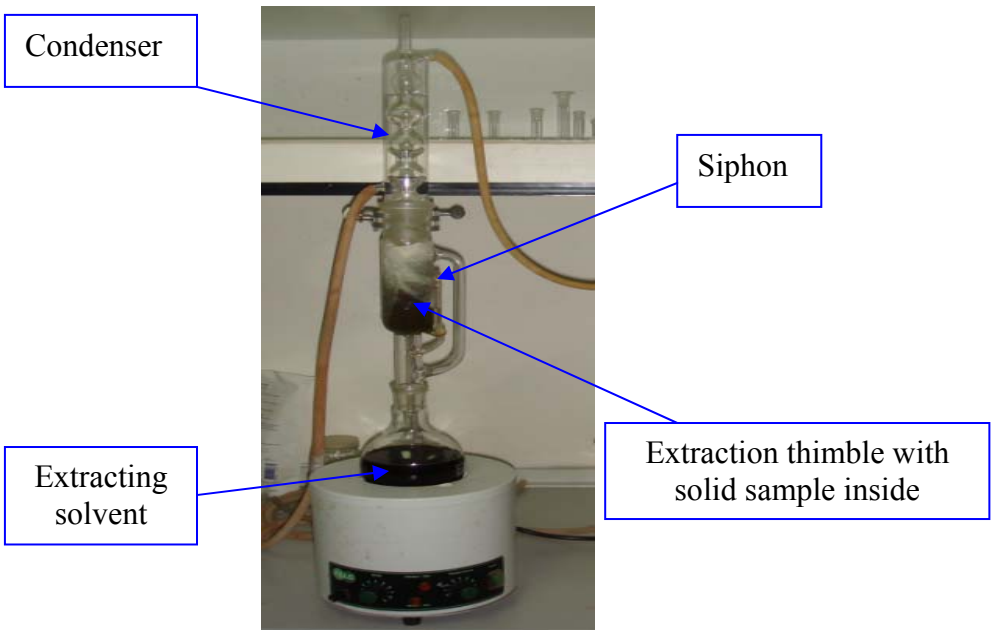


Figure 2.1 Soxhlet extraction apparatus

2.3.2 Chromatographic methods

Chromatography is the method of choice in solving the problem of isolation of a compound of interest from a complex natural mixture. There are various methods from basic to advance, supporting efficient isolation and separation of compounds.

2.3.2.1 Thin-layer chromatography (TLC)

TLC is an easy, cheap, rapid, and basic method for the analysis and isolation of natural and synthetic compounds. It involves the use of particulate adsorbent spreaded on inert sheet of glass, plastic, or metal as a stationary phase. The mobile phase is allowed to travel up the plate carrying the sample that was initially spotted on the adsorbent just above the solvent. Depending on the nature of the stationary phase, the separation can be either partition (C18 reversed-phase) or adsorption chromatography (silica gel, alumina, cellulose, and polyamide). The advantage of TLC is that samples do not have to undergo expensive pre-cleanup

steps, and it has the ability to detect a wide range of compounds, using reactive spray reagents. Non-destructive detection (fluorescent indicator in the plates and examination under a UV lamp) also makes it possible for purified samples to be scraped off the plate and be analyzed by other techniques (Leven, Vanden-Berghe, Mertens, Vilietick, and Lammens, 1978; Hamburger, Cordell, Tantivana, and Ruangrungsi, 1987).

2.3.2.2 Preparative TLC (PTLC)

Prep-TLC has long been a popular method as a primary or final purification step in an isolation procedure. Separation can be effected rapidly and amount of material isolated is from 1 mg to 1 g. The adsorbent thickness of PTLC is 0.5-4 mm whereas that of analytical TLC is 0.1-0.2 mm. For commercially available PTLC plates, adsorbents (silica, alumina, C18, and cellulose) are usually 0.5, 1.0, and 2.0 mm in thickness (Breitmaier, 2002).

2.3.2.3 Antioxidant TLC bioassays

In addition to the simplicity and the ability of TLC to separate mixtures quickly and more economically, it can be readily used to detect biological activity of separated components. Currently, TLC bioassays are used more and more widely.

Antioxidant substances are very important and valuable components for human health. Therefore, identifying their presence in the mixture as easily and quickly as possible is convenient for further experimentation. To investigate the antioxidant, $DPPH^{\bullet}$ (2,2'-diphenyl-1-picrylhydrazyl) radical is used. The $DPPH^{\bullet}$ is a stable radical, and in the presence of radical scavengers, it converts from purple to yellow as shown in Figure 2.2 (Owen *et al*., 2003).

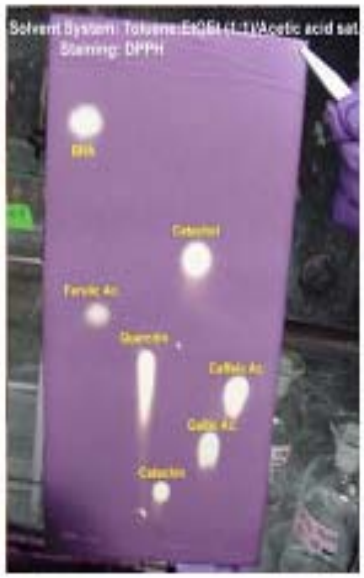


Figure 2.2 Some antioxidants identified by antioxidant TLC assay

The antioxidant activity is examined by running a TLC plate with samples, with concentration ranging from 0.1 to 100 μg. The plate is dried, sprayed with a DPPH$^{\bullet}$ solution (2 mg/mL in methanol), and left for half an hour. Antioxidant compounds appear as yellow spots against a purple background (Breitmaier, 2002).

2.3.2.4 Column chromatography (CC)

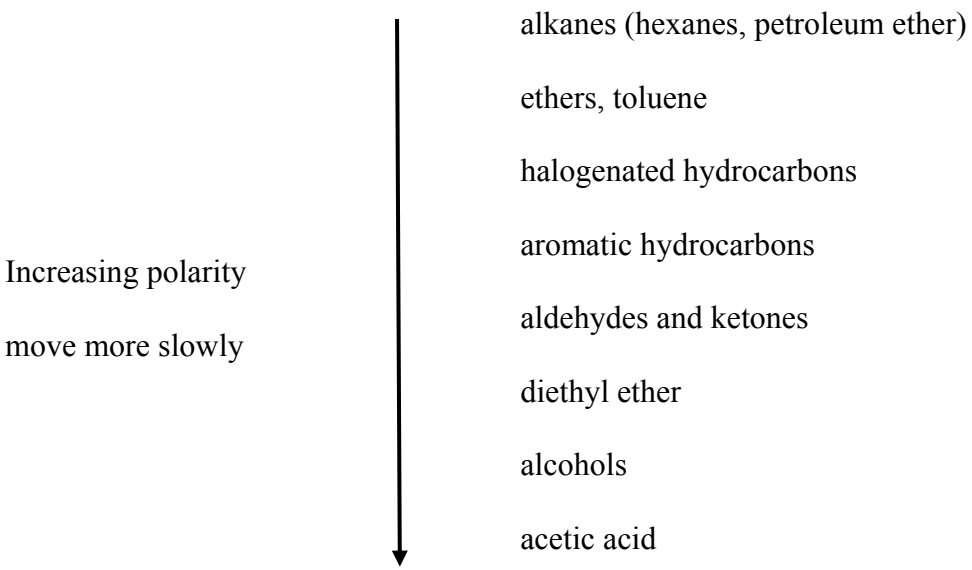
In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass (usually) column and the mobile phase, a liquid, is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique to isolate desired compounds from a mixture. The mixture to be analyzed by column chromatography is applied to the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components

in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or eluents, are collected as the solvent drips from the bottom of the column. The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents. Consequently, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column. Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique. TLC is generally used to determine the system for a column chromatography separation. The scheme 2.1 shows the expected elution order of organic compounds with different functional groups (Pine, 1987).

To determine best system for developing TLC plate or chromatography column loaded with an unknown mixture, the polarity of the solvent is varied in several trial runs and the results of the chromatography are carefully observed and recorded in each solvent system, when increasing the polarity of the solvent system, all the components of the mixture move faster. The ideal solvent system is simply the system that separates the components.

TLC elution patterns usually extrapolate to column chromatography elution patterns. Since TLC is a much faster procedure than column chromatography, TLC is often used to determine the best solvent system for column chromatography.

For instance, in determining the solvent system for a flash chromatography procedure, the ideal system is the one that moves the desired component of the mixture to a TLC R_f value of 0.25-0.35 and will separate this component from its nearest neighbor by difference in R_f values of at least 0.20. Therefore, a mixture is analyzed by TLC to determine the ideal solvent(s) for a flash chromatography procedure.



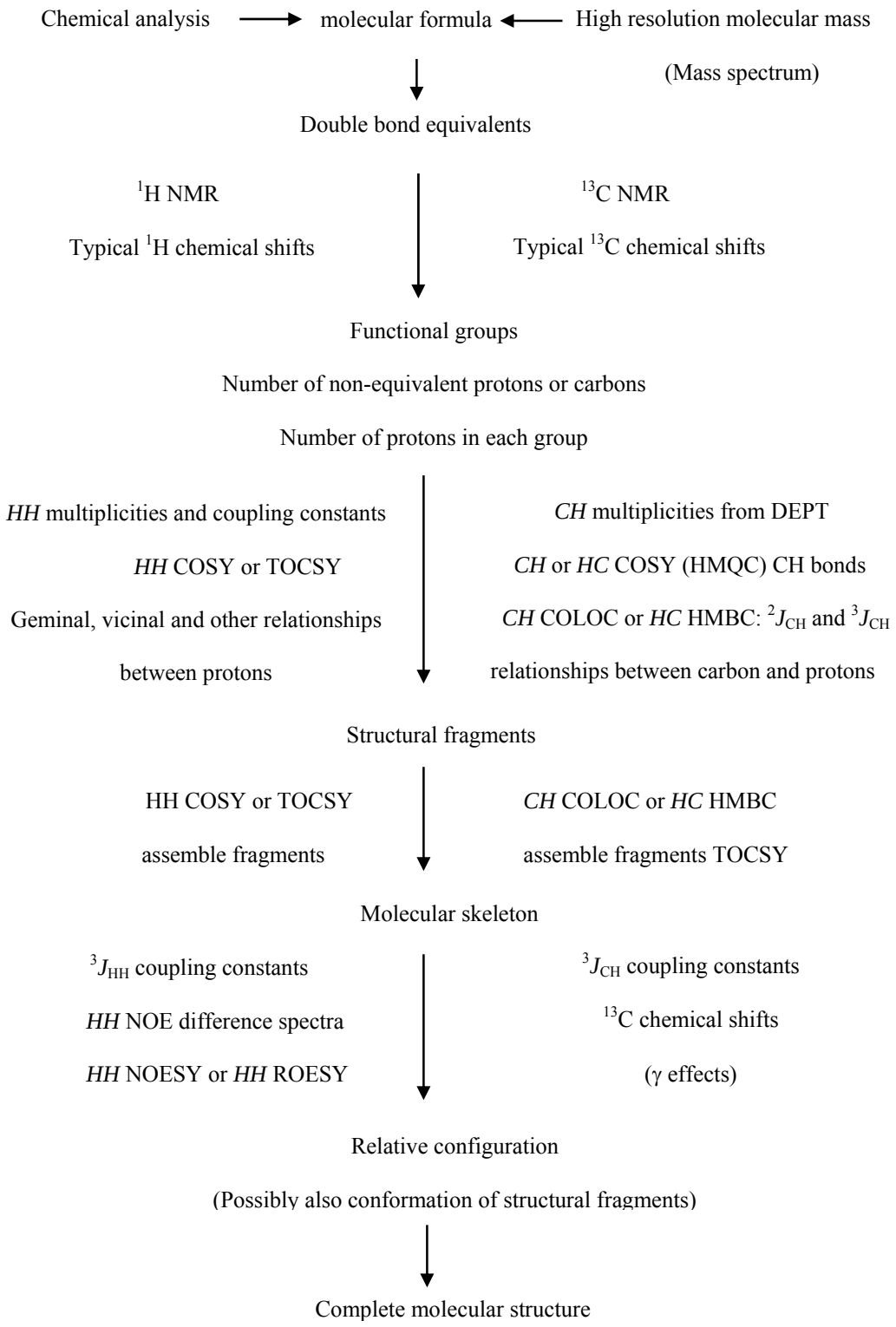
Scheme 2.1 The expected elution order of organic classes

2.3.3 Structure elucidation

2.3.3.1 Nuclear magnetic resonance (NMR) spectroscopy

NMR has become a very important spectroscopic method and the premier spectroscopy available to organic chemists to determine the detailed chemical structure of the chemicals they are isolating from natural products. NMR spectroscopy is routinely used by chemists to study chemical structure of simple molecules using simple one-dimensional techniques (1D-NMR). Two-dimensional techniques (2D-NMR) are used to determine the structure of more complicated

molecules. The following are suggested tactics for solving structure using NMR (Breitmaier, 2002).



Scheme 2.2 Suggested tactics for solving structure using NMR

One dimensional NMR

1-D NMR was the only NMR based technique used until the 1970's, *i.e*. the spectrum has 2 axes, plotting frequency against intensity. Some of these techniques, often employing complex pulse sequences to obtain specific information, are still very useful in determining structures from spectra. Some of these are summarized below (Ngan, 2005):

Table 2.1 Multiple pulse 1-D NMR techniques.

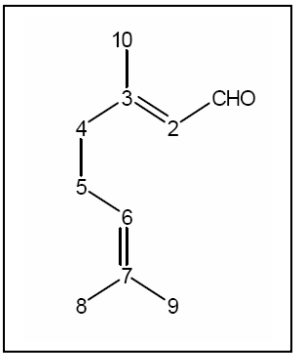
Experiment	Nuclei Observed	Types of Information, Applications
J-modulated spin-echo (attached proton test, APT)	^{13}C	CH and CH_3 carbon nuclei give positive signals. 4° and CH_2 carbons give negative signals.
INEPT	^{1}H /^{13}C	The INEPT pulse sequence is used as a component of many 2-D experiments e.g. HSQC.
DEPT	^{13}C	Tells how many hydrogen atoms are directly bonded to a carbon nucleus: CH, CH_2, CH_3. Disadvantage: no signals from 4° carbon atoms.
Selective TOCSY	^{1}H	Allows identification of all the protons belonging to a common coupled spin system.

Table 2.1 (Continued)

Experiment	Nuclei Observed	Types of Information, Applications
1D-INADEQUATE	^{13}C	Exact ^{13}C-^{13}C coupling constants without the need to synthesize ^{13}C enriched compounds.

Proton NMR (^{1}H-NMR)

The area under the plots provides information about the number of protons present in the molecule, the position of the signals (the chemical shift) reveals information regarding the chemical and electronic environment of the protons, and the splitting pattern provides information about the number of neighboring (vicinal or geminal) protons. The abscissa shows the chemical shift (δ) values of the different type of protons and the ordinate shows the intensities of the signals. For instance, proton NMR data (Figure 2.3) of a monoterpene, citral ($C_{10}H_{16}O$), isolated from lemon grass oils. The signals of protons attached to saturated carbon atoms such as methyl, methylene, as well as methine groups appear between δ 0.8 and 2.4 ppm. The most intense peaks arise from the methyl groups. The less intense peaks arise from both the methylene as well as the methine groups. The signals between 4.8 and 5.9 ppm correspond to the olefinic methine groups. Furthermore, the signal from 9.0 to 10.0 ppm is typical for aldehyde groups shown in Figure 2.3 (Ngan, 2005).



^{1}H; 1.65 (*d*), 2.15 (*s*), 2.11 (*m*), 2.24 (*m*), 5.00(*d*), 5.80(*d*), 9.84(*d*)

Figure 2.3 Structure and chemical shifts of citral

Carbon NMR (^{13}C-NMR)

Similar to proton NMR, carbon NMR is a plot of signals arising from the different ^{13}C-NMR experiments as a function of chemical shift. The signals appear as singlets because of the decoupling of the attached protons. Different techniques of recording of the ^{13}C-NMR have been developed so that it is possible to differentiate between the various types of carbons such as the primary, secondary, tertiary and quaternary carbons. The range of the chemical shift values in the ^{1}H-NMR (normally 0-12 ppm) differs from those in the ^{13}C-NMR (normally 0-230 ppm, and this wide range allows the peaks to be more widespread making structure elucidation easier). The difference arises from the two nuclei having different numbers of electrons around their corresponding nuclei as well as different electronic configurations. The most important ^{13}C-NMR for determining multiplicities of carbon atoms is Distortionless Enhancement by Polarization Transfer (DEPT). This technique determines the number of hydrogen attached to a given carbon. In DEPT-45, only carbon atom that bears attached hydrogen(s) will produce a peak. DEPT-90 shows peaks only for carbon atoms that are a part of a methine (CH) group. In DEPT-

135, methine and methyl carbons give positive peaks, whereas methylene carbons appear as inverse peaks (Ngan, 2005).

2.3.3.2. Infrared spectroscopy (IR)

IR is used to probe bond vibrations in molecules. This is a simple method to reveal the types of functional groups present in compound. Functional group region in the range from 4000-1600 cm^{-1} can be divided into three bands:

- OH, NH, C-H
- C≡C, C≡N, X=C=Y (X, Y = C, O, N, S)
- C=O, C=C

The finger print region has very little information about functional group and is used only to compare two compounds. The range is 1550-660 cm^{-1}.

IR spectra can be measured either as solution (in $CHCl_3$) or in the solid state mixed with KBr or as thin liquid film. The range of measurement is from 4000-660 cm^{-1} and frequency is from 6 x 10^{11} to 4000 x 10^{11} Hz. A simplified correlation table is given in Table 2.2 (Lambert *et al*., 1987).

Table 2.2 A simplified correlation of infrared absorption with organic functional groups.

Functional Group	Frequency (cm^{-1})	Intensity	Assignment
Alkanes must have	3000-2800	s	-C-H Stretch
both peaks	1500-1450	s	
Alkenes must have	3100-3000	m	=C-H Stretch
both peaks	1675-1600	m	C=C Stretch

Table 2.2 (Continued)

Functional Group	Frequency (cm^{-1})	Intensity	Assignment
Alkynes must have	3300-3200	w	C-H Stretch
both peaks	2200-2100	w	≡C-C Triple bond stretch
Alcohols, phenols	3600-3000	broad	O-H Stretch
	1300-1000		C-O Stretch
Aldehydes must have	1750-1625	s	C=O Stretch
all three peaks	2850-2800	w	C-H Stretch of C=O
	2750-2700	w	C-H Stretch of C=O
Aromatic rings (i.e.	3100-3000	w	=C-H Stretch
benzene rings) must	1600-1580	m	C=C Stretch
have all three peaks	1500-1450	m	C=C Stretch
Ketones	1750-1625	s	C=O Stretch
Carboxylic acids	3400-2400	very broad	H-Bonded O-H stretch
must have all three			across the entire range
peaks	1730-1660	s	C=O Stretch
	1320-1210	s	C-O Stretch
Ethers	1300-1000	s	C-O Stretch
Amines: primary	3500-3200	m	N-H Stretch (two peaks
must have both peaks	1640-1560	s	in this range)
			N-H Bend

Table 2.2 (Continued)

Functional Group	Frequency (cm^{-1})	Intensity	Assignment
Amines: secondary	3500-3200	m	N-H Stretch (one peak
must have both peaks	1550-1450	s	in this range)
Nitriles	2300-2200	m	N-H Bend
Nitro groups	1600-1500	m	C-N Triple bond
must have both peaks	1400-1300	s	stretch
	(2 peaks < 200		N=O Stretch
	cm^{-1} apart)		N=O Stretch
Amides	3500-3100	m	N-H Stretch
must have all three	1670-1600	s	C=O Stretch
peaks	1640-1550	m	N-H Bend

Intensity Key: s = strong, m = medium, w = weak

2.3.3.3 Ultraviolet (UV) spectroscopy

Frequency of ultraviolet/visible is 7.5 x 10^{14} to 300 x 10^{14} Hz, which probes outer core electron transitions. The absorption spectrum is measured in dilute solution.

There are three factors for a good solvent. Firstly, the dilute solvent must not absorb the UV radiation in the same region as the substance whose spectrum is being determined. Secondly, the solvent should not affect the fine structure of an absorption band. The last factor is the ability of the solvent to influence the wavelength of UV light that will be absorbed via stabilization of either the ground or the excited state (Lambert *et al*., 1987).

The maxima and minima of the absorption are recorded in nm and the intensity is recorded in terms of log ε with ε = A/c.l (A: absorbance, c: molar concentration, l: length of sample cell).

It is useful to identify conjugated system of the compound. The 95% ethanol is the common solvent used for UV. UV is used in identification by measuring the solution as neutral and by adding basic and acidic solution to the unknown or under different pH. The change of the maxima to higher or lower λ_{max} indicates the class of compounds. The value of UV and visible spectra in identifying unknown constituents is indicative of the compounds (Lambert *et al*., 1987).

These some following observations can be used to identify functional groups, double bonds, aromatics systems, etc.

•Two bands of medium intensity (ε = 1,000 to 10,000), both with λ_{max} above 200 nm indicated the presence of an aromatic system.

•A single band of low intensity (ε = 10 to 100) in the region 250 to 360 nm, with no major absorption at shorter wavelength (200 to 250 nm) usually indicates a $n \rightarrow \pi^*$ transition. Such compound contains O, N, or S atom and may include C=O, C=N, N=N, NO_2, -COOR, and –COOH (Lambert *et al*., 1987).

2.3.3.4 Mass Spectrometry (MS)

Mass spectrometry is an analytical technique used to measure the mass-to-charge ratio of ions. This is a powerful, sensitive, and highly selective method to identify compounds. It provides both molecular weight and fragmentation pattern of a compound. It relies on production of ions from a parent compound and the subsequent characterization of the patterns that are produced. Mass spectrometers can be divided into three fundamental parts, namely the ionization source, the

analyzer, and the detector (Lambert *et al*., 1987; Hughes, Croley, Metcalfe, and March, 2001; Proestos, Sereli, and Komaitis, 2006).

The sample has to be introduced into the ionization source of the instrument. Once inside the ionization source, the parent compound is bombarded by high-energy electron stream and then converted to ions, because ions are easier to manipulate than neutral molecules. These ions are extracted into the analyzer region of the mass spectrometer where they are separated according to their mass-to-charge ratios (m/z) in a magnetic or electric field. The separated ions are detected by a detector and this signal is sent to a data system where the m/z ratios are stored together with their relative abundance for presentation in the format of an m/z spectrum (Lambert *et al*., 1987; Hughes *et al*., 2001; Proestos *et al*., 2006).

Molecular ions and fragment ions are accelerated by manipulation of the charged particles through the mass spectrometer. Uncharged molecules and fragments are pumped away. These are some of the different types of mass analyzer (Lambert *et al*., 1987; Hughes *et al*., 2001; Proestos *et al*., 2006).

Table 2.3 Some kinds of mass analyzer.

Analyzer	**System Highlights**
Quadrupole mass filter and quadrupole ion storage (ion trap)	Unit mass resolution, fast scan
Sector (magnetic and/or electrostatic); double focusing	High resolution, exact mass
Time-of-flight (TOF)	Theoretically, no limitation for m/z maximum, high throughput

Table 2.3 (Continued)

Analyzer	**System Highlights**
Ion cyclotron resonance (ICR)	Very high resolution, exact mass

MS is combined with GC or LC (HPLC) to form a remarkable analytical technique, which is used widely to separate a mixture and identify spectroscopy of compounds on-line (Lambert *et al*., 1987; Hughes *et al*., 2001; Proestos *et al*., 2006).

CHAPTER III

EXPERIMENTAL

Unless indicated otherwise, all experiments were performed at Suranaree University of Technology.

3.1 Source of plant materials

Fresh rhizomes of greater galangal were purchased from Suranakorn fresh market, located in Muang District, Nakhon Ratchasima province, Thailand in August and November 2005, and June and December 2006. Fresh rhizomes of smaller galangal were digged from Saengduan Konekratoke's paddy field located in Chokchai District, Nakhon Ratchasima province in July and December 2005, and June and November 2006. The specimens were authenticated by Dr. Paul J. Grote, School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima province.

3.2 Chemical sources

Commercial grade hexane, chloroform, and methanol were obtained from Fluka. Analytical grade chloroform and methanol, and concentrated sulfuric acid were purchased from J. T Baker. HPLC grade methanol, deuterochloroform, and deuteromethanol were obtained from Aldrich. Silica gel 60 Art. 7734 and 9385, silica gel 60 G Art. 7731 and GF_{254} Art. 7730, silica gel 60 F_{254} precoated TLC aluminum

sheet (20 x 20 cm^2 in size with layer thickness of 0.2 mm), vanillin, and KBr were purchased from Merck. Standard galangin was obtained from Indofine Chemical Company, Inc. Dragendorff's reagent and ferric chloride reagent were provided by Aussavashai Shuyprom, a previous graduate student, School of Chemistry, Institute of Science, Suranaree University of Technology.

3.3 Chemical purity and preparation

Hexane, chloroform, and methanol used for extraction and elution were of commercial grade and were distilled prior to use. Methanol used for mass analyses was of HPLC grade, KBr for FT-IR experiments was of IR grade, chloroform and methanol used for recrystallization, and all other chemicals were of analytical grade and were used without additional purification.

Dragendorff's reagent was prepared by Aussavashai Shuyprom by adding 10 mL of 40% aqueous solution of potassium iodide (KI) to 10 mL of solution of 0.85 g of basic bismuth subnitrate ($Bi_5O(OH)_9(NO_3)_4$) in 10 mL of acetic acid and 50 mL of distilled water, and diluting the resulting solution with acetic acid and water in the ratio of 1:2:10. Vanillin reagent was prepared by dissolving 4 g of vanillin in 100 mL of concentrated sulfuric acid. Ferric chloride reagent was prepared by Aussavashai Shuyprom by dissolving 12.5 g of ferric chloride in 250 mL of 0.5 N hydrochloric acid.

Preparative TLC plates for final separation were prepared as follows. A mixture of 200 g of silica gel 60 GF_{254} in 480 mL water was shaken thoroughly until the suspension was homogeneous. It was allowed to stand in a stoppered flask for 1-2 hours before being spreaded onto eight 20 x 20 cm^2 glass plates with the layer

thickness of 2 mm. The plates were left to dry in still air for 24 hours and in an oven at 100 °C for 1 hour.

3.4 Instrumentation

The dired rhizomes were ground by using an Otto blender. The removal of a large quantity of volatile solvent from extraction and separation processes was carried out by using a Buchi R-114 rotary evaporator and a water pump. The UV-visible spectra were recorded on a Hewlett Packard 8452A diode array UV-vis spectrophotometer, and FT-IR spectra on a Perkin-Elmer spectrophotometer (Model Spectrum GX). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-400 NMR spectrophotometer and mass spectra on a Hewlett Packard 5989 HP mass spectrometer at Department of Chemistry, Mahidol University, Bangkok.

3.5 Plant material preparation

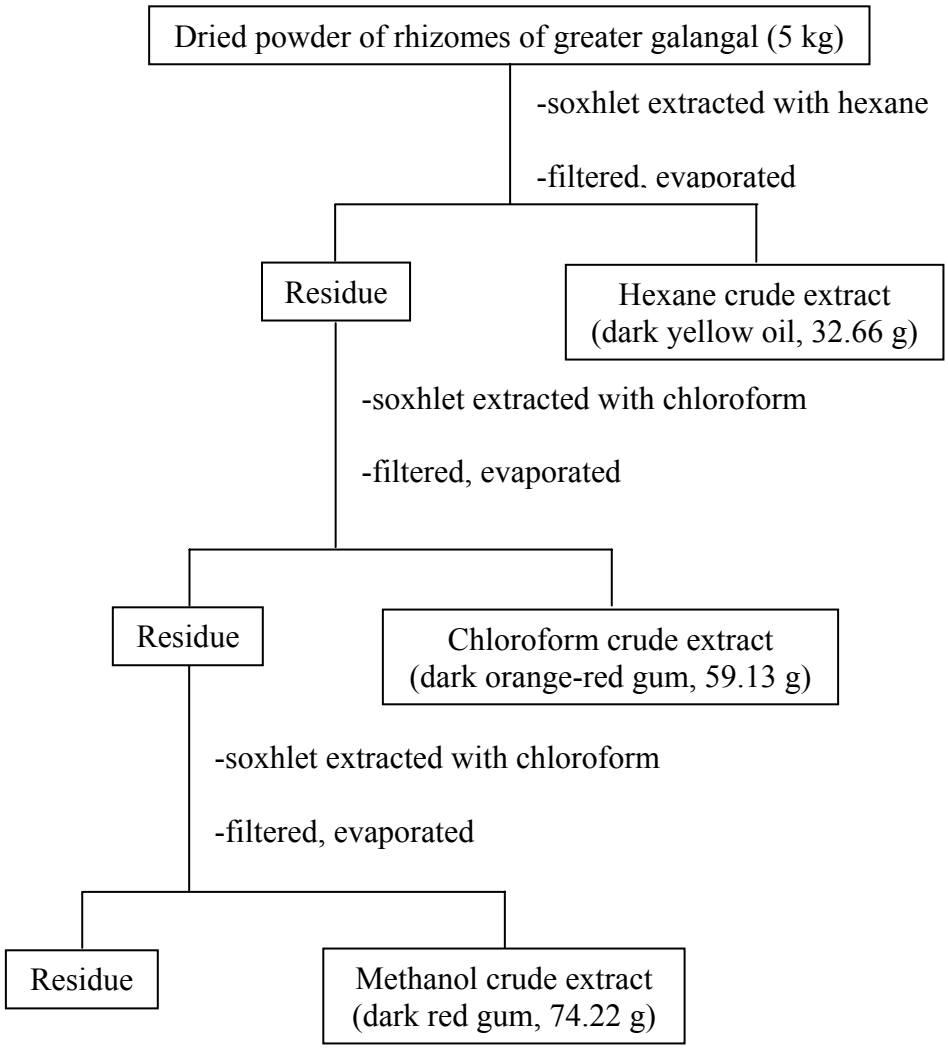
The rhizomes of both greater and smaller galangal were separated from the stems, washed thoroughly, and dried in an oven at 50 °C for three days. The dried samples were then ground to powder.

3.6 Extraction

3.6.1 Greater galangal

The five kilograms of dried powder of rhizomes of greater galangal were extracted consecutively with hexane by soxhlet extraction apparatus for 12 hours, chloroform for 12 hours, and methanol for 12 hours. The extracted solutions were then filtered through a filter paper (Whatman No. 1). The filtrates were concentrated

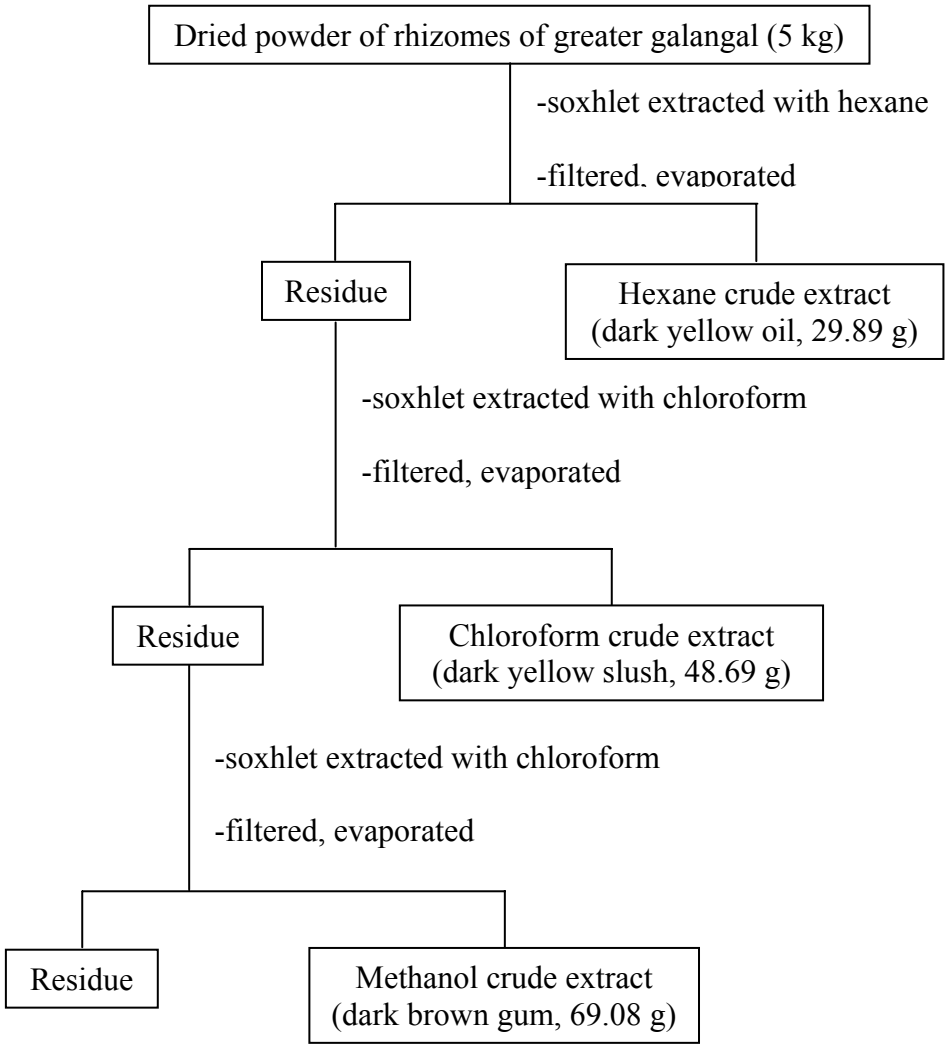
by evaporation under reduced pressure to afford 32.66 g of hexane crude extract as dark yellow oil, 59.13 g of chloroform crude extract as dark orange-red gum, and 74.22 g of methanol crude extract as dark red gum. The extraction sequence is shown in Scheme 3.1.



Scheme 3.1 Extraction of the rhizomes of greater galangal

3.6.2 Smaller galangal

The five kilograms of dried powder of rhizomes of smaller galangal were extracted in the same manner as those of grater galangal to give 29.89 g of hexane crude extract as dark yellow oil, 48.69 g of chloroform crude extract as dark yellow slush, and 69.08 g of methanol crude extract as dark brown gum. The extraction sequence is shown in Scheme 3.2.



Scheme 3.2 Extraction of the rhizomes of smaller galangal

3.7 Isolation

3.7.1 Greater galangal

The hexane crude extract was discarded because it contained nonpolar compounds which were not interesting. The chloroform and methanol crude extracts were examined by TLC (2 x 5 cm^2 in size with chloroform: methanol, 9:1 and 8:2 as developing solvents) to find galangin by comparison with the standard galangin. The resulting spots were visible as quenching spots under UV light at 254 nm and no galangin were found in both crude extracts. However, the chloroform crude extract was further separated using a column (5 cm in diameter and 45 cm in length) packed with hexane slurry of silica gel 60 Art. 7734 to the height of 35 cm. The crude extract was dissolved in a small amount of chloroform and loaded onto the top of the column. The column was then eluted successively with hexane, hexane: chloroform (1:1), chloroform, chloroform: methanol (1:1), and methanol. Every fraction of 250 mL was collected and concentrated to a small volume and four major fractions (I 5.59 g, II 6.41 g, III 10.93 g, and IV 17.74 g) were separated by monitoring with TLC (2 x 5 cm^2 in size with chloroform: methanol, 9:1 as developing solvent) in order to combine the fractions with the same compounds. Each fraction was then examined by TLC (4 x 5 cm^2 in size with chloroform: methanol, 9:1 as developing solvent) by comparison with the standard galangin under UV light at 254 nm. No galangin was found.

3.7.2 Smaller galangal

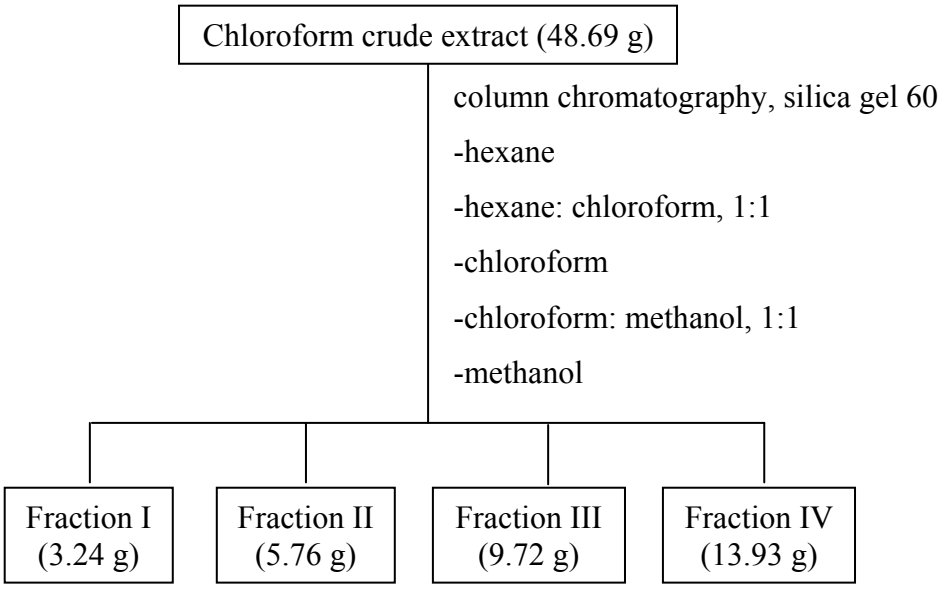
The hexane crude extract was discarded because it contained nonpolar compounds which were not interesting. The chloroform and methanol crude extracts were examined by TLC (2 x 5 cm^2 in size with chloroform: methanol, 9:1 and 8:2 as developing solvents) to find galangin by comparison with the standard galangin.

Under UV light at 254 nm, a spot with the same position as the standard galangin appeared in chloroform crude extract as quenching spot. Three tests of spot detection were performed to confirm the presence of galangin in the crude extracts. With Dragendorff's reagent, the spots with the same position as the standard galangin and standard galangin itself turned to black color after heating at 80-100 °C for a few minutes. With vanillin reagent, the spots with the same position as the standard galangin and standard galangin itself turned to yellow color after heating at 100 °C for a few minutes. With ferric chloride reagent, the spots with the same position as the standard galangin and standard galangin itself turned to dark brown color after heating at 80-100 °C for a few minutes. However, it was not clear whether galangin was in the methanol crude extract due to poor separation on the TLC plates.

The chloroform crude extract was further separated using a column (3 cm in diameter and 30 cm in length) packed with hexane slurry of silica gel 60 Art. 7734 to the height of 20 cm. The crude extract was dissolved in a small amount of chloroform and loaded onto the top of the column. The column was then eluted successively with hexane, hexane: chloroform (1:1), chloroform, chloroform: methanol (1:1), and methanol. Every fraction of 250 mL was collected and concentrated to a small volume and four major fractions (I 3.24 g, II 5.76 g, III 9.72 g and IV 13.93 g) were separated by monitoring with TLC (2 x 5 cm^2 in size with chloroform: methanol, 9:1 as developing solvent) in order to combine the fractions with the same compounds. The separation sequence is shown in Scheme 3.3.

After examining each fraction with TLC (4 x 5 cm^2 in size with chloroform: methanol, 9:1 as developing solvent) by comparison with the standard galangin under

UV light at 254 nm. There was no galangin in fraction I and II, and spots of galangin together with other compounds were found in fraction III and IV.

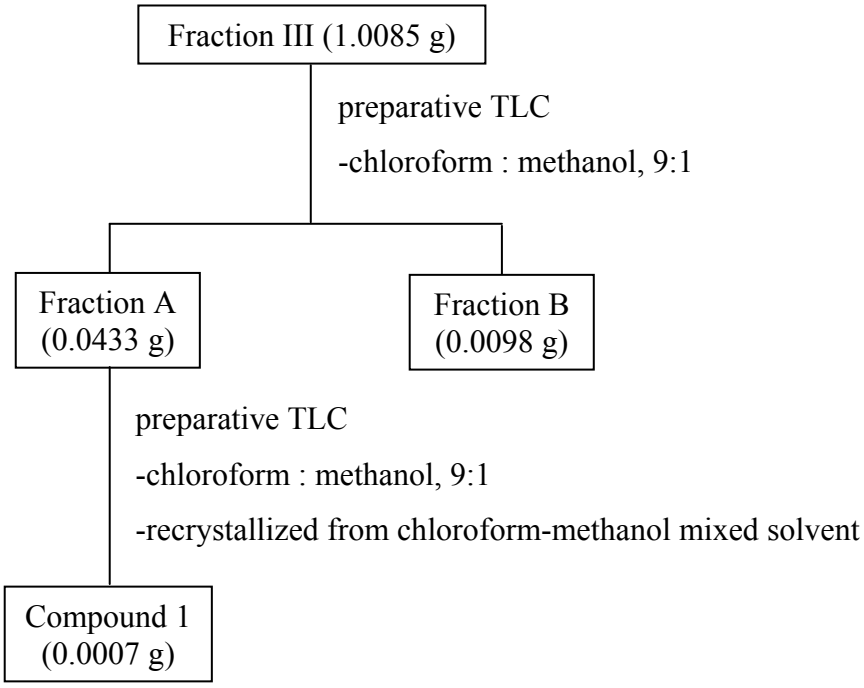


Scheme 3.3 Separation of chloroform crude extract of smaller galangal

A portion of fraction III (1.0085 g) was further separated by preparative TLC (20 x 20 cm^2 in size, coated with methanol suspension of silica gel 60 G Art. 7731 with layer thickness of 2 mm and chloroform: methanol, 9:1 as developing solvent). Two fractions (A 0.0433 g and B 0.0098 g) were afforded. Fraction A was further purified by preparative TLC (the plate preparation was mentioned in page 45) using the same developing solvent to give crude compound 1 (0.0105 g), which was recrystallized from chloroform-methanol mixed solvents to obtain pure compound 1 (0.0007 g) as light yellow powder. The isolation sequence of compounds 1 is shown in Scheme 3.4.

Fraction IV (13.93 g) was further separated using a column (3 cm in diameter and 30 cm in length) packed with hexane slurry of silica gel 60 Art. 7731 to

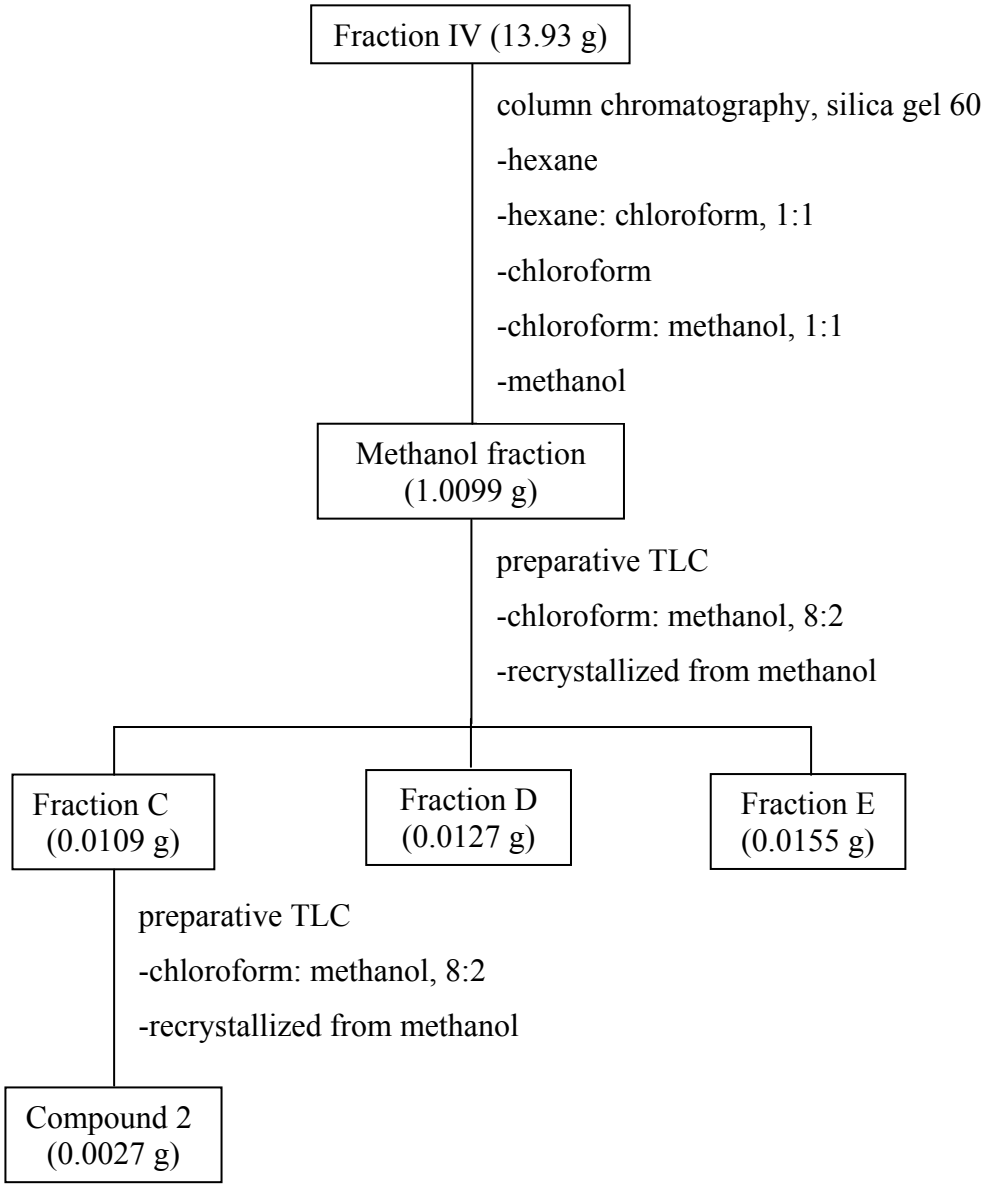
the height of 20 cm. The column was eluted successively with hexane, hexane: chloroform (1:1), chloroform, chloroform: methanol (1:1), and methanol. Every fraction of 100 mL was collected and concentrated to a small volume. A portion of methanol fraction (1.0099 g) was further separated by preparative TLC (20 x 20 cm^2 in size, coated with methanol suspension of silica gel 60 G Art. 7731 with layer thickness of 2 mm and chloroform: methanol, 8:2 as developing solvent) to afford three fractions (C 0.0109 g, D 0.0127 g, and E 0.0155 g).



Scheme 3.4 Isolation of compound 1 from fraction III of chloroform crude extract

Fraction C (0.0109 g) was further purified by preparative TLC (the plate preparation was mentioned in page 45) using the same developing solvent to give crude compound 2 (0.0069 g) which was recrystallized from methanol to obtain pure

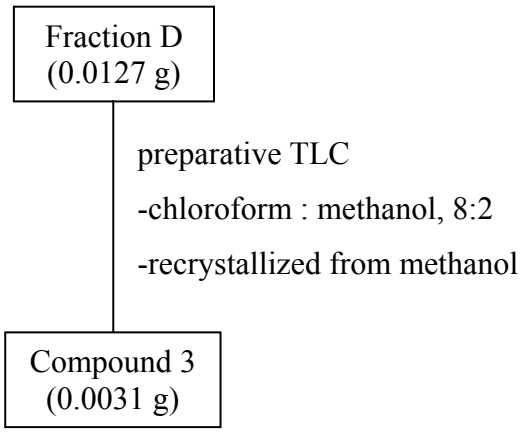
compound 2 (0.0027 g) as light yellow powder. The isolation sequence of compound 2 is shown in Scheme 3.5.



Scheme 3.5 Isolation of compound 2 from fraction IV of chloroform crude extract

Fraction D (0.0127 g) was further purified by preparative TLC (the plate preparation was mentioned in page 45) using the same developing solvent to give

crude compound 3 (0.0103 g), which was recrystallized from methanol to obtain pure compound 3 (0.0031 g) as bright yellow powder. The isolation sequence of compound 3 is shown in Scheme 3.6.



Scheme 3.6 Isolation of compounds 3 from fraction D of chloroform crude extract

The methanol crude extract was separated in the same manner as the chloroform crude extract to give four fractions (I 3.18 g, II 5.77 g, III 12.22 g, and IV 16.86 g). However, after examining with TLC, no galangin was found in any fractions.

3.8 Characterization of the isolated compounds from smaller galangal

3.8.1 Compound 1

UV ($CHCl_3$) λ_{max} values are 267 and 370 nm (Figure A.1).

FT-IR spectrum ν_{max} (KBr) values are 3607-3084, 1659, 1600, 1550 and 1260 cm^{-1} (Table 4.1 and Figure A.2).

^{1}H-NMR δ (ppm) values, in CD_3OD are 12.43 (1H, *s*, 5-OH), 10.74 (1H, *s*, 7-OH), 9.59 (1H, *s*, 3-OH), 8.20 (2H, *dd*, 2', 6'-H), 7.62 (2H, *dd*, 3', 5'-H), 7.53 (1H,

m, 4'-H), 6.43 ($J_{H8/H6}$= 1.5 Hz, *d*, 1H, 8-H), 6.21 (1H, *d*, 6-H) (Table 4.2 and Figure A.3).

^{13}C-NMR δ (ppm) values, in CD_3OD, are 178.1 (4-C), 169.1 (5-C), 166.2 (7-C), 159.7 (9-C), 159.8 (2-C), 136.4 (3-C), 130.2 (1'-C), 128.5 (3', 5'-C), 127.9 (4'-C), 126.1 (2', 6'-C), 103.2 (10-C), 98.1 (6-C), 97.8 (8-C) (Table 4.3 and Figure A.4).

3.8.2 Compound 2

UV ($CHCl_3$) λ_{max} values are 272, 315 and 378 nm (Figure A.6).

FT-IR spectrum ν_{max} (KBr) values are 3650-3300, 2950, 2875, 1640, 1610, 1425 and 1125 cm^{-1} (Table 4.4 and Figure A.7).

^{1}H-NMR δ (ppm), in CD_3OD, are 12.33 (1H, *s*, 5-OH), 10.81 (1H, *s*, 7-OH), 9.41 (1H, *s*, 3-OH), 8.07 (2H, *d*, 2', 6'-H), 7.05 (2H, *d*, 3', 5'-H), 6.41 (1H, *d*, 8-H), 6.25 (1H, *d*, 6-H), 3.79 (3H, *s*, 4'-OCH_3) (Table 4.5 and Figure A.8).

^{13}C-NMR δ (ppm) values, in CD_3OD are 176.0 (4-C), 163.7 (7-C), 160.5 (5-C), 160.3 (4'-C), 156.1 (9-C), 146.0 (2-C), 135.9 (3-C), 129.2 (2', 6'-C), 123.0 (1'-C), 114.0 (3', 5'-C), 103.4 (10-C), 98.1 (6-C), 93.3 (8-C), 55.1 (4'-OCH_3) (Table 4.6 and Figure A.9).

3.8.3 Compound 3

FT-IR spectrum ν_{max} (KBr) values are 3650-3200, 2970, 2825, 1639, 1625 and 1200 cm^{-1} (Table 4.7 and Figure A.10).

^{1}H-NMR δ (ppm), in CD_3OD, are 12.41 (1H, *s*, 5-OH), 10.69 (1H, *s*, 7-OH), 8.03 (2H, *d*, *J* = 8.72 Hz, 2^1, 6^1), 7.01 (2H, *d*, *J* = 8.72 Hz, 3^1, 5^1), 5.95 (1H, *s*, H-6), 5.37 (1H, *d*, *J* = 7.90 Hz, 1^2-H), 3.80 (3H, *s* , 4^1- OMe), 3.21 (1H, *m*, 2^2-H), 3.30 (1H, *m*, 3^2-H), 3.13 (1H, *m*, 4^2-H), 3.42 (1H, *m*, 5^2, 6^2-H) (Table 4.8 and Figure A.11).

^{13}C-NMR δ ppm, in CD_3OD, are 176.84 (4-C), 163.6 (7-C), 160.6 (4^1-C), 160.6 (5-C), 155.7 (2-C), 155.3 (9-C), 133.0 (3-C), 130.2 (2^1,6^1-C), 122.0 (1^1-C), 113.3 (3^1,5^1-C), 103.7 (10-C), 100.4 (1^2-C), 98.2 (6-C), 93.3 (8-C), 76.4 (5^2-C), 76.0 (3^2-C), 73.8 (2^2-C), 69.4 (4^2-C), 60.5 (6^2-C), 55.0 (4-OMe) (Table 4.9 and Figure A.12).

CHAPTER IV

RESULTS AND DISCUSSION

The characterization of the structures of the isolated compounds was on the basis of NMR spectra by comparison of their NMR spectral data with literature values. Details on structure determination of chemical constituents will be discussed in Chapter IV.

4.1 Structural elucidation of compound 1

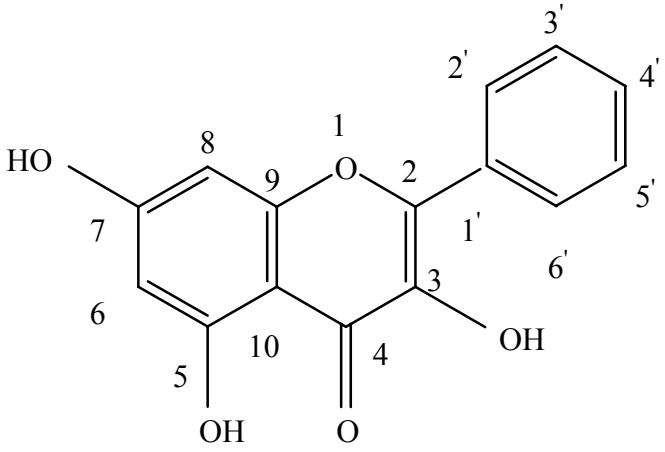


Figure 4.1 Structure of compound 1

The characteristic light yellow and the UV spectrum (Figure A.1) of compound 1 suggested that compound 1 is a flavonoid derivative compound (Maria *et al*., 2007).

The IR spectrum of compound 1 is shown in Figure A.2 and important absorptions were attributable to hydroxyl (3607-3084 cm^{-1}) and carbonyl functions (1659 cm^{-1}). Other peaks were assigned as shown in Table 4.1.

Table 4.1 IR absorption band assignment of compound 1

Wave number (cm^{-1})	**Intensity**	**Assignment**
3607-3084	s	O–H Stretch
1659	m	C=O Stretch
1600,1550	m	C=C Stretch
1260	m	C–H Bend

s = strong, m = medium

The ^{1}H-NMR (Figure A.3) and ^{13}C-NMR (Figure A.4) spectra of compound 1 showed the necessary diagnostic peaks to be identified as a flavonoid derivative with hydroxyl group on C-3, C-5 and C-7, appearing as sharp singlets at δ 9.59, 12.43 and 10.74 ppm, respectively. The H-6 and H-8 occurred as sharp doublet at δ 6.21 and 6.43 ppm, respectively ($J_{H8/H6}$ = 1.53 Hz). The B ring signals were easily assigned by consideration of symmetry. The H-2' and H-6' resonances occurred as sharp doublet at δ 8.20 ppm. The H-3' and H-5' resonances appeared as doublet of doublets at δ 7.62 ppm and H-4' occurred as muiltplet at δ 7.53 ppm. The ^{13}C experiments of compound 1 gave thirteen peaks. The most downfield shifted peak was 178.1 ppm which was assigned as ketone group (C-4).

Compound 1 (Figure 4.1) exhibited the ^{1}H-NMR and ^{13}C-NMR chemical shifts identical to 3,5,7-trihydroxyflavone (galangin) as shown in Table 4.2 and 4.3 with

comparison of its spectral data with previous reports (Rubens and Wagner, 2005). It's exactly attested by the structure of galangin.

Table 4.2 The ^{1}H-NMR chemical shifts of compound 1$^{\#}$ and galangin*

Proton Position	**Chemical Shifts in ppm (δ_H)**	
	Compound 1	**Galangin**
3-OH	9.59 (*s*)	9.61 (*s*)
5-OH	12.43 (*s*)	12.52 (*s*)
6	6.21 (*d*)	6.23 (*d*)
7-OH	10.74 (*s*)	10.79 (*s*)
8	6.43 (*d*)	6.45 (*d*)
2'	8.20 (*dd*)	8.32 (*dd*)
3'	7.62 (*dd*)	7.64 (*dd*)
4'	7.53 (*m*)	7.55 (*m*)
5'	7.62 (*dd*)	7.64 (*dd*)
6'	8.20 (*dd*)	8.32 (*dd*)

$^{\#}$400 MHz ^{1}H, CD_3OD
*500 MHz ^{1}H, CD_3OD

Table 4.3 The ^{13}C-NMR chemical shifts of compound 1$^{\#}$ and galangin*

Carbon Position	**Chemical Shifts in ppm (δ_C)**	
	Compound 1	**Galangin**
2	159.8	160.0
3	136.4	136.6
4	178.1	178.3
5	169.1	169.3

Table 4.3 (Continued)

Carbon Position	Chemical Shifts in ppm (δ_C)	
	Compound 1	**Galangin**
6	98.1	98.3
7	166.2	166.4
8	97.8	98.0
9	159.7	160.1
10	103.2	105.5
1'	130.2	130.4
2'	126.1	126.4
3'	128.5	128.7
4'	127.9	128.0
5'	128.5	128.7
6'	126.1	126.4

[#]125 MHz ^{13}C, pyridine d_5
[*]125 MHz ^{13}C, pyridine d_5

Further studies of the structure of compound 1 were done by analysis of the high resolution EI-MS. The MS spectra (Figure A.5) showed the $[M+H^+]$ and $[M-H^-]$ peaks at m/z = 271 and 269, respectively.

4.2 Structural elucidation of compound 2

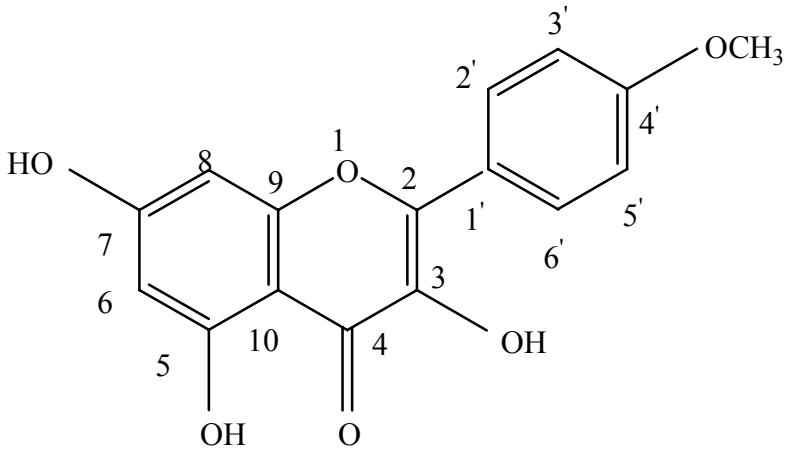


Figure 4.2 Structure of compound 2

The characteristic light yellow and the UV spectrum (Figure A.6) of compound 2 suggested that compound 2 is a flavonoid derivative compound similar to compound 1 (Maria *et al*., 2007).

The IR spectrum of compound 2 is shown in Figure A.7 and important absorptions were attributable to hydroxyl (3650-3300 cm^{-1}) and carbonyl (1640 cm^{-1}) groups. Peaks at 2950 and 2875 cm^{-1} were assigned as sp^3 C–H, 1640 cm^{-1} as C=O, 1610 and 1425 cm^{-1} as conjugated C=C aromatic ring, and 1125 cm^{-1} as C–O as shown in Table 4.4.

Table 4.4 IR absorption band assignment of compound 2

Wave number (cm^{-1})	**Intensity**	**Assignment**
3650-3300	S	O–H Stretch
2950, 2875	m	C–H Stretch
1640	s	C=O Stretch

Table 4.4 (Continued)

Wave number (cm^{-1})	Intensity	Assignment
1610, 1425	s	C=C Stretch
1125	m	C–O Bend

s = strong, m = medium

The ^{1}H-NMR (Figure A.8) and ^{13}C-NMR (Figure A.9) spectra of compound 2 showed the necessary diagnostic peaks to be identified as a flavonoid derivative with hydroxyl group on C-3, C-5 and C-7, appearing as sharp singlets at δ 9.41, 12.33 and 10.81 ppm, respectively. The H-6 and H-8 occurred as sharp doublet at δ 6.25 and 6.41 ppm, respectively. The B ring signals were easily assigned by consideration of symmetry. The H-2' and H-6' occurred as sharp doublet at δ 8.07 ppm. The H-3' and H-5' appeared as doublet at δ 7.05 ppm, and 4'-OCH_3 occurred as a singlet at δ 3.79 ppm. The ^{13}C experiments of compound 2 gave fourteen peaks. The most downfield shifted peak was 176.0 ppm which was assigned as ketone group (C-4) and most upfield shifted peak was 55.1 ppm which was assigned as ether group (4'-OCH_3).

Compound 2 (Figure 4.2) exhibited the ^{1}H-NMR and ^{13}C-NMR chemical shifts identical to 3,5,7-trihydroxy-4'-methoxyflavone (kaempferide) as shown in Table 4.5 and 4.6 with comparison of its spectral data with previous reports (Eunjung *et al*., 2008). It's exactly attested by the structure of kaempferide.

Table 4.5 The ^{1}H-NMR chemical shifts of compound 2$^{\#}$ and kaempferide*

Proton	**Chemical Shifts in ppm (δ_H)**	
Position	**Compound 2**	**Kaempferide**
3-OH	9.41 (*s*)	9.47 (*s*)
5-OH	12.33 (*s*)	12.43 (*s*)
6	6.25 (*d*)	6.29 (*d*)
7-OH	10.81 (*s*)	10.83 (*s*)
8	6.41 (*d*)	6.45 (*d*)
2'	8.07 (*d*)	8.12 (*d*)
3'	7.05 (*d*)	7.09 (*d*)
4'-OCH_3	3.79 (*s*)	3.81 (*s*)
5'	7.05 (*d*)	7.09 (*d*)
6'	8.07 (*d*)	8.12 (*d*)

$^{\#}$400 MHz ^{1}H, pyridine d_5
*400 MHz ^{1}H, DMSO d_6

Table 4.6 The ^{13}C-NMR chemical shifts of compound 2$^{\#}$ and kaempferide*

Carbon	**Chemical Shifts in ppm (δ_H)**	
Position	**Compound 2**	**Kaempferide**
2	146.0	146.3
3	135.9	136.1
4	176.0	176.1
5	160.5	160.8
6	98.1	98.3
7	163.7	164.1

Table 4.6 (Continued)

Carbon Position	Chemical Shifts in ppm (δ_H)	
	Compound 2	**Kaempferide**
8	93.3	93.6
9	156.1	156.3
10	103.4	103.7
1'	123.0	123.3
2'	129.2	129.4
3'	114.0	114.1
4'	160.3	160.6
5'	114.0	114.1
6'	129.2	129.4
4'-OCH_3	55.1	55.4

[#]125 MHz ^{13}C, pyridine d_5
[*]125 MHz ^{13}C, DMSO d_6

4.3 Structural elucidation of compound 3

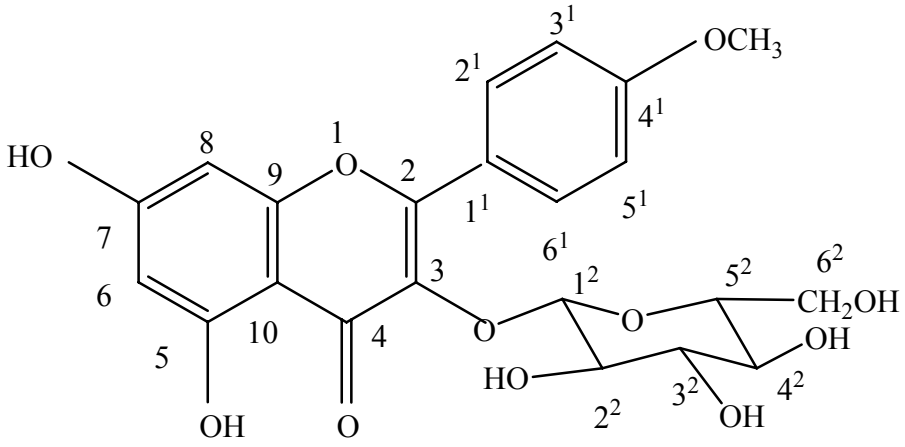


Figure 4.3 Structure of compound 3

The characteristic bright yellow suggested that compound 3 is a flavonoid glucoside derivative compound (Paolo, Marcello, Virginia, and Orazio, 2001; Xu, Liu, Li,Wu, and Liu, 2008).

The IR spectrum of compound 3 is shown in Figure A.10, quite similar to that of compound 2 and important absorptions were attributable to hydroxyl (3650-3200 cm^{-1}) and carbonyl functions (1639 cm^{-1}). Peaks at 2970 and 2825 cm^{-1} were assigned as sp^3 C–H, 1639 cm^{-1} as C=O, 1625 and 1500 cm^{-1} conjugated C=C aromatic ring, and 1200 cm^{-1} as C–O as shown in Table 4.7.

Table 4.7 IR absorption band assignment of compound 3

Wave number (cm^{-1})	**Intensity**	**Assignment**
3650-3200	s	O–H Stretch
2970, 2825	m	C–H Stretch
1639	m	C=O Stretch

Table 4.7 (Continue)

Wave number (cm^{-1})	Intensity	Assignment
1625, 1500	s	C=C Stretch
1200	m	C–O Bend

s = strong, m = medium

The ^{1}H-NMR (Figure A.11) and ^{13}C-NMR (Figure A.12) spectra of compound 3 showed that all the glucose units had β-linkages to either another glucose or glycone since the constant coupling between the anomeric protons and 2^{2}-H were always 7-8 Hz and anomeric carbon resonances were in the δ 100 ppm region (Agrawal, 1992). The ^{13}C NMR spectral data suggested that glucose in compound 3 was attached to the flavonol aglycone at C-3, because of the lack of downfield shift for C-3 and the presence of a hydrogen bonding with the carbonyl group in position 4 at δ 176.84 ppm.

Compound 3 exhibited the ^{1}H-NMR and ^{13}C-NMR chemical shifts identical to kaempferide-3-*O*-*β*-D-glucoside (Juha-Pekka *et al*., 2004). A comparison of the ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 3, kaempferide-3-*O*-*β*-D-glucoside and kaempferol-3-*O*-*β*-D-glucoside (Eunjung *et al*., 2008) is shown in Table 4.8 and 4.9. These data indicated that compound 3 is kaempferide-3-*O*-*β*-D-glucoside (Figure 4.3).

Table 4.8 The ^{1}H-NMR chemical shifts of compound 3[#], and kaempferide-3-*O*-*β*-D-glucoside[#] and kaempferol-3-*O*-*β*-D-glucoside[*]

Carbon Position	**Chemical Shifts in ppm (δ_H)**		
	Compound 3	**Kaempferide-3-*O*-*β*-D-glucoside**	**Kaempferol-3-*O*-*β*-D-glucoside**
5-OH	12.41 (*s*)	12.45 (*s*)	12.53 (*s*)
6	5.95 (*s*)	5.98 (*s*)	6.11 (*s*)
7-OH	10.69 (*s*)	10.79 (*s*)	10.81 (*s*)
8	6.07 (*s*)	6.18 (*s*)	6.23 (*s*)
2^1	8.03 (*d*, *J* = 8.72 Hz)	8.09 (*d*, *J* = 8.96 Hz)	8.14 (*d*, *J* = 8.80 Hz)
3^1	7.01 (*d*, *J* = 8.72 Hz)	7.05 (*d*, *J* = 8.96 Hz)	7.09 (*d*, *J* = 8.80 Hz)
4^1-OCH_3	3.80 (*s*)	3.83 (*s*)	-
5^1	7.01 (*d*, *J* = 8.72 Hz)	7.05 (*d*, *J* = 8.96 Hz)	7.09 (*d*, *J* = 8.80 Hz)
6^1	8.03 (d, *J* = 8.72 Hz)	8.09 (d, *J* = 8.96 Hz)	8.14 (*d*, *J* = 8.80 Hz)
1^2-glu	5.37 (*d*, *J* = 7.90 Hz)	5.40 (*d*, *J* = 7.90 Hz)	5.42 (*d*, *J* = 7.90 Hz)
2^2	3.21 (*m*)	3.30 (*m*)	3.33 (*m*)
3^2	3.30 (*m*)	3.35 (*m*)	3.41 (*m*)
4^2	3.13 (*m*)	3.18 (*m*)	3.25 (*m*)
5^2	3.42 (*m*)	3.45 (*m*)	3.53 (*m*)
6^2	3.42 (*m*)	3.45 (*m*)	3.54 (*m*)

[#]400 MHz ^{1}H, CD_3OD
[*]400 MHz ^{1}H, DMSO, d_6

Table 4.9 The ^{13}C-NMR chemical shifts of compound 3[#], kaempferide-3-*O*-*β*-D-glucoside[#] and kaempferol-3-*O*-*β*-D-glucoside[*]

Carbon Position	**Chemical Shifts in ppm (δ_C)**		
	Compound 3	**Kaempferide-3-*O*-*β*-D-glucoside**	**Kaempferol-3-*O*-*β*-D-glucoside**
2	155.7	155.92	155.87
3	133.0	133.06	132.76
4	176.8	176.94	176.91
5	160.6	160.66	159.42
6	98.2	98.37	98.33
7	163.6	163.66	163.60
8	93.3	93.35	93.30
9	155.3	155.35	155.75
10	103.7	103.71	103.65
1^1	122.0	122.09	120.49
2^1	130.2	130.29	130.44
3^1	113.3	113.32	114.70
4^1	160.6	160.70	160.71
5^1	113.3	113.32	114.70
6^1	130.2	130.29	130.44
4^1-OMe	55.0	55.15	-
1^2	100.4	100.48	100.34
2^2	73.8	73.89	73.67

Table 4.3 (Continued)

Proton Position	Chemical Shifts in ppm (δ_C)		
	Compound 3	**Kaempferide-3-*O*-*β*-D-glucoside**	**Kaempferol-3-*O*-*β*-D-glucoside**
3^2	76.0	76.11	75.98
4^2	69.4	69.63	69.36
5^2	76.4	77.23	76.95
6^2	60.5	60.61	60.31

[#]125 MHz ^{13}C, pyridine d_5
[*]100 MHz ^{13}C, DMSO d_5

CHAPTER V

CONCLUSION

The dried powder of the rhizomes of smaller galangal was extracted with hexane, chloroform, and methanol respectively. The chloroform crude extract was isolated by column chromatography and preparative thin-layer chromatography to give three pure compounds. The chemical structures were characterized on the basis of NMR spectral analysis, including ^{1}H-NMR and ^{13}C-NMR in comparison with literature values. The structures of three isolated compounds are summarized as follows.

Compound 1 was identified as 3,5,7-trihydroxyflavone (galangin). The ^{1}H and ^{13}C-NMR spectroscopic evidence revealed the presence of the characteristic flavonol group. The amount of pure compound 1 obtained was at least 0.0007 g.

Compound 2 was identified as 3,5,7-trihydroxy-4'-methoxyflavone (kaempferide). The ^{1}H and ^{13}C-NMR spectra of compound 2 were similar to those of compound 1, except the presence of extra three CH_3 protons on the B ring. The amount of pure compound 2 obtained was at least 0.0027 g.

Compound 3 was identified as 5,7-dihydroxy-4'-methoxy-3-*O*-*β*-D-glucopyranosideflavone (kaempferide-3-*O*-*β*-D-glucoside). The amount of pure compound 3 obtained was at least 0.0031 g. The ^{1}H and ^{13}C-NMR spectra of compound 3 were in agreement with those obtained from the literature.

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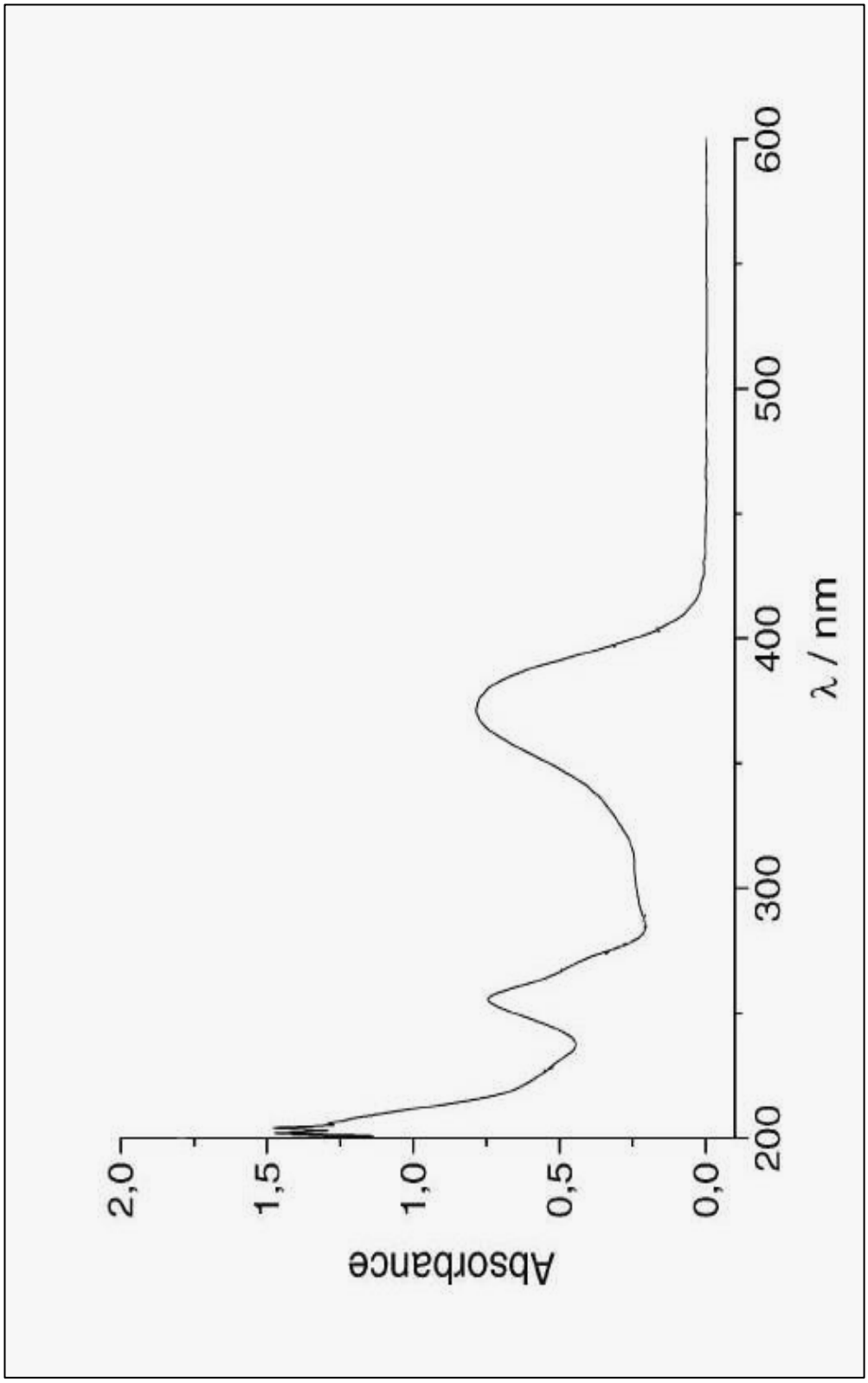
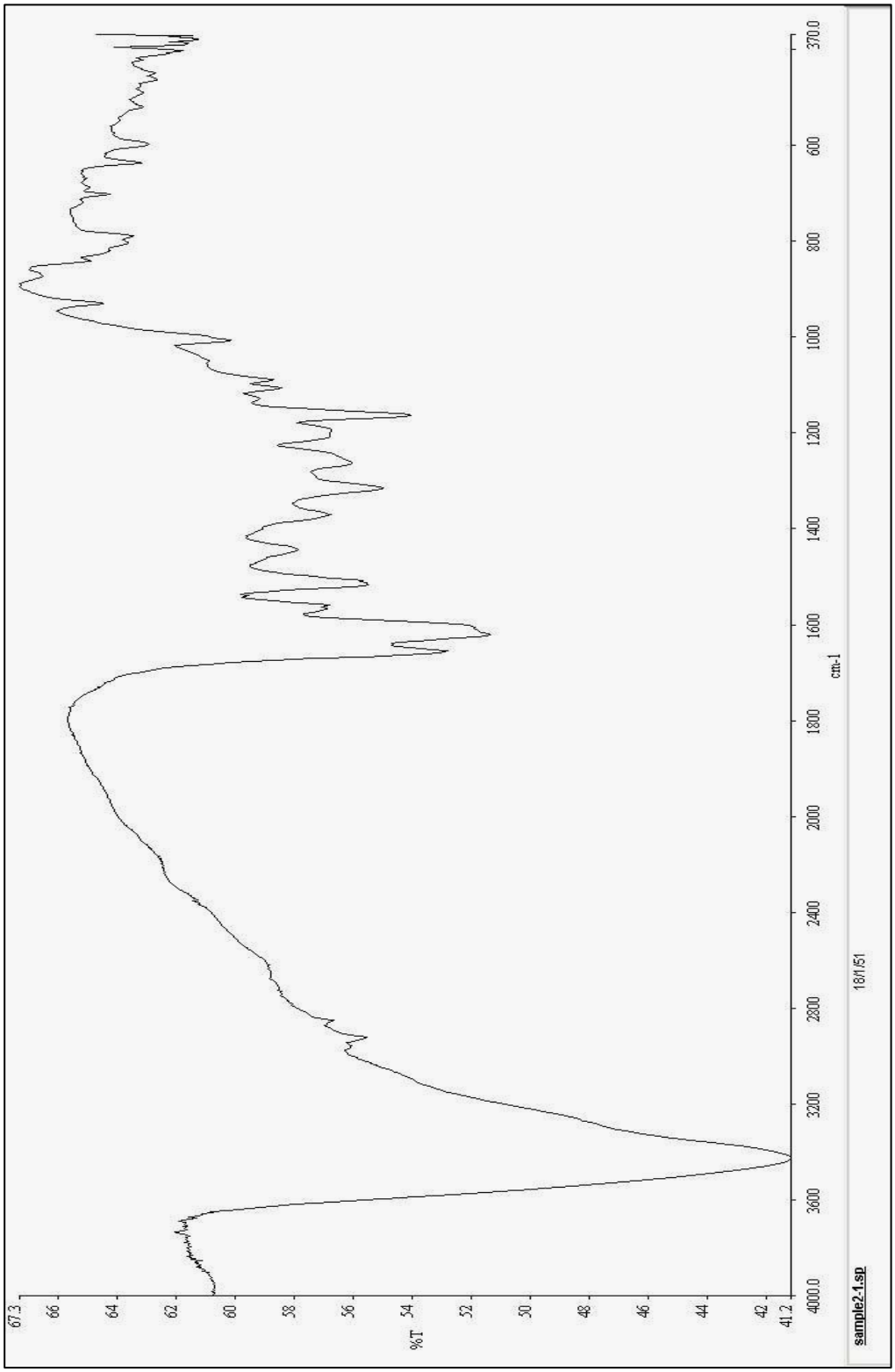
APPENDIX

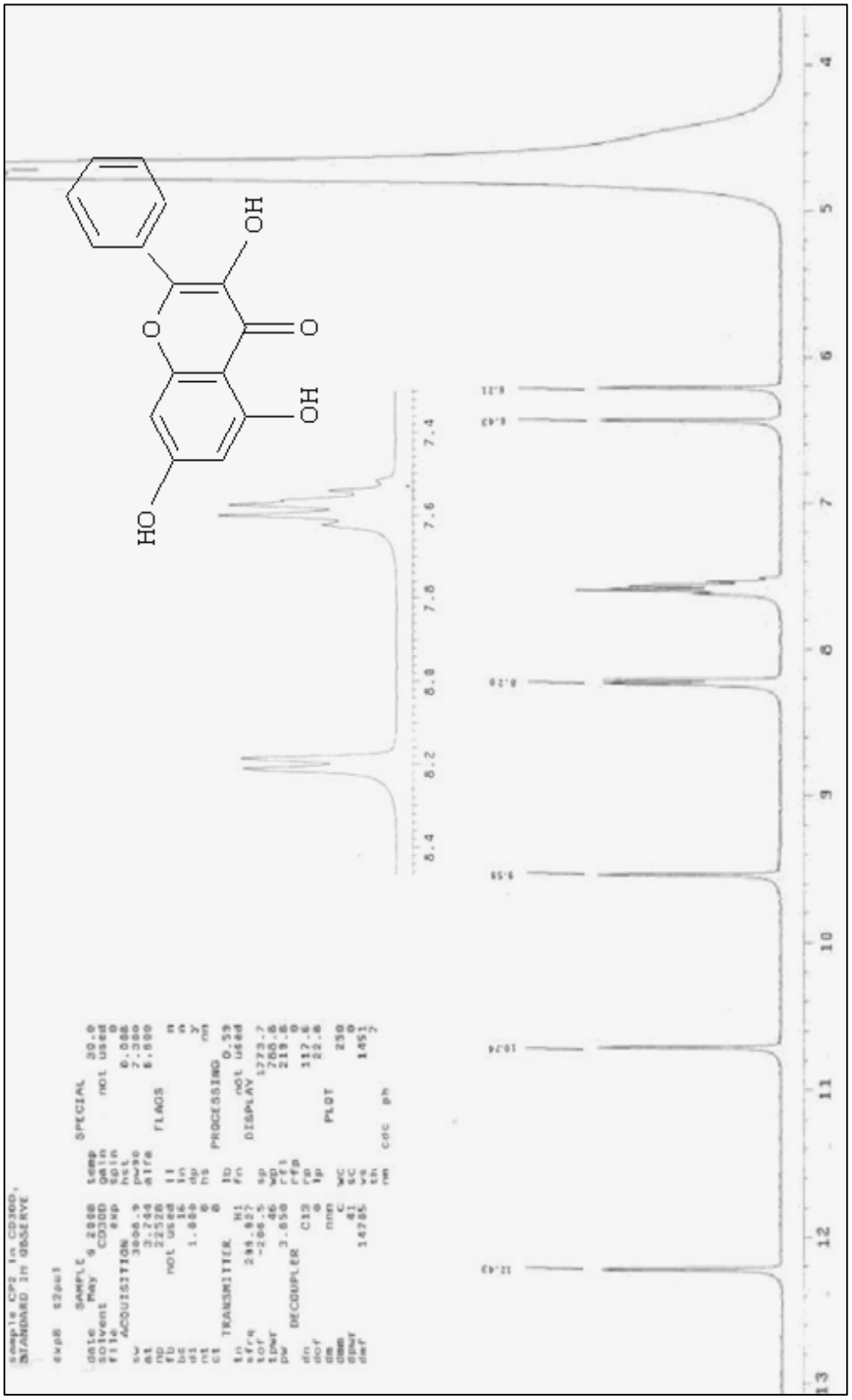
A.1 UV-Vis spectrum of compound 1



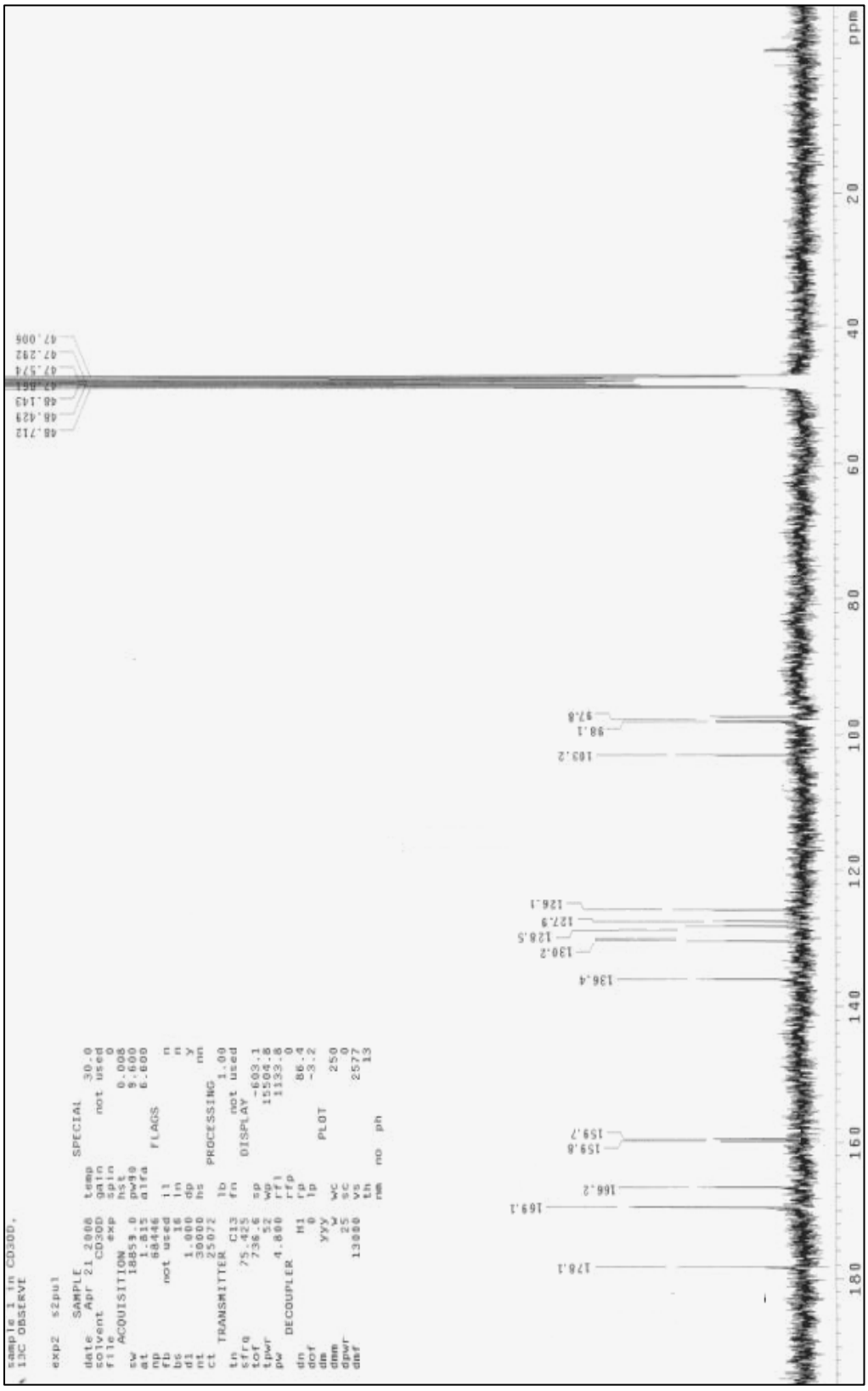
A.2 IR spectrum of compound 1



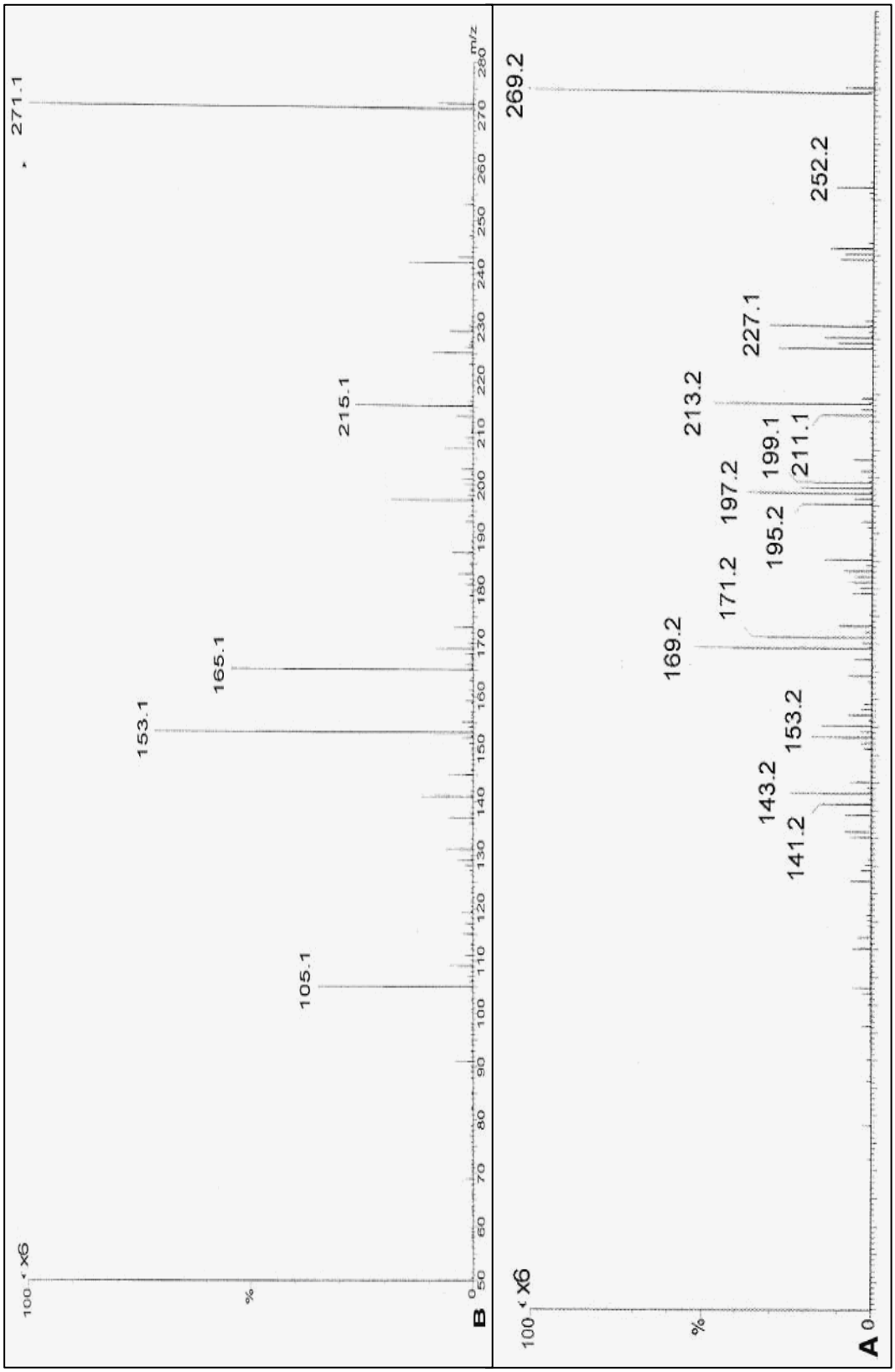
A.3 ^{1}H-NMR spectrum of compound 1



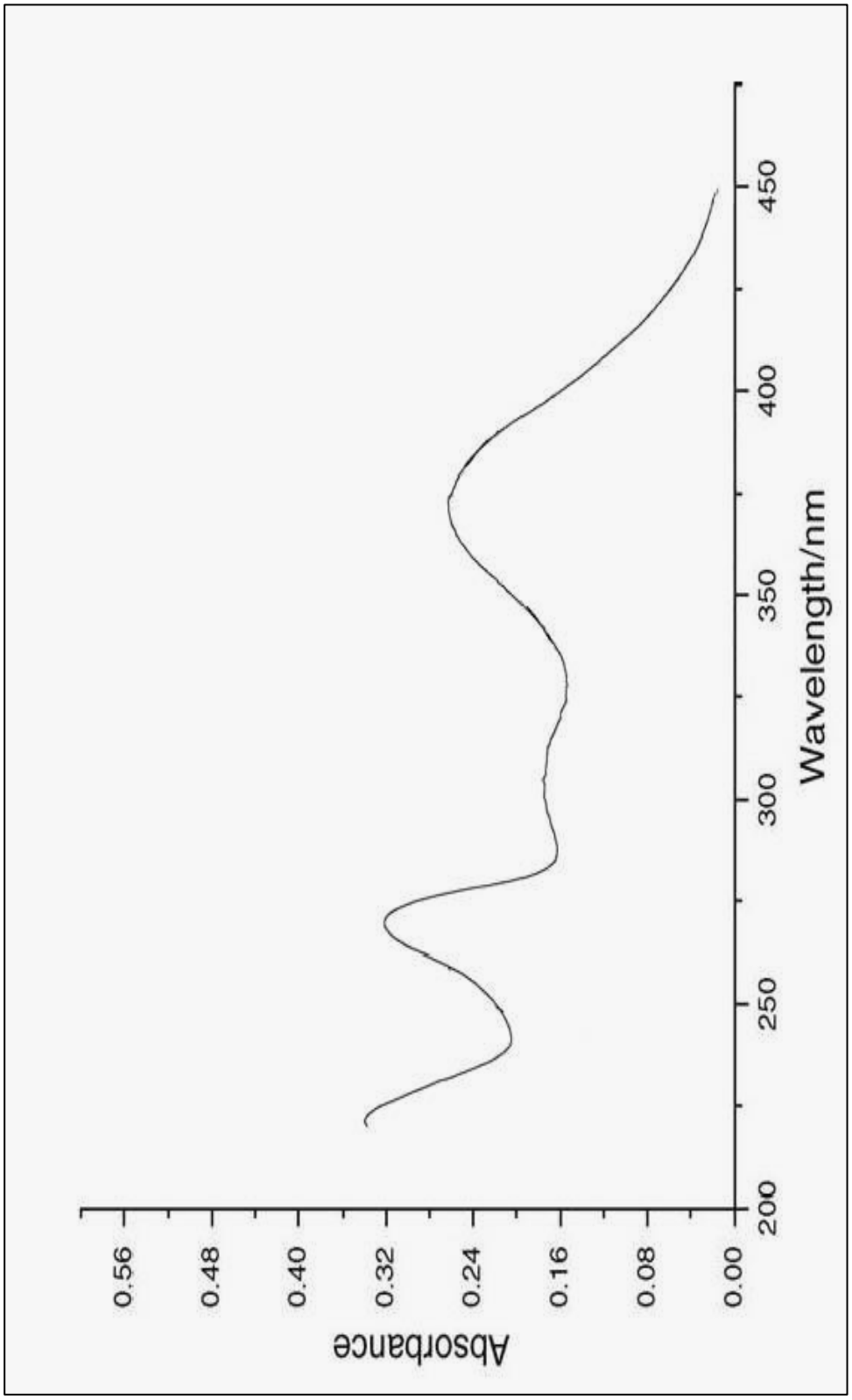
A.4 ^{13}C-NMR spectrum of compound 1



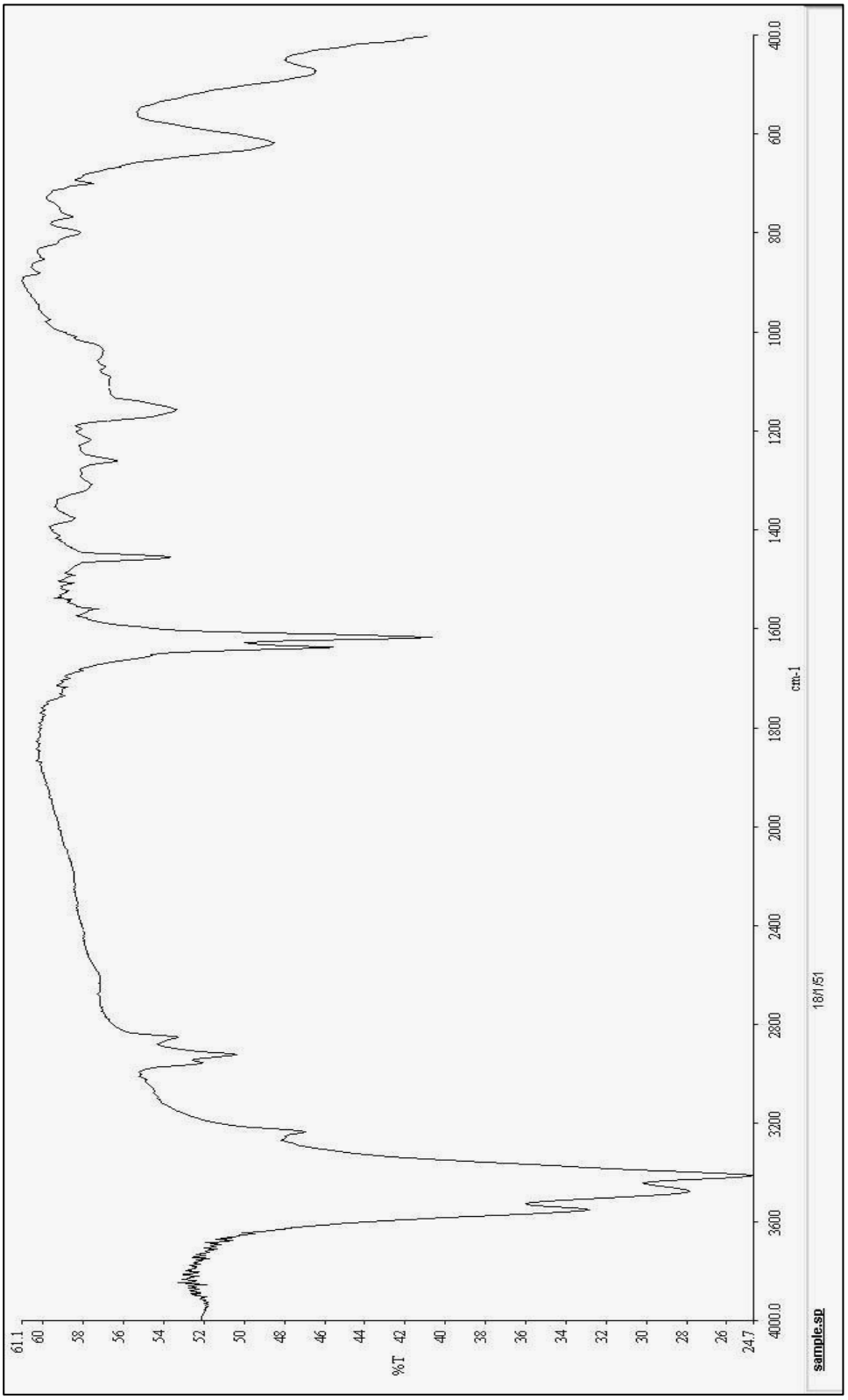
A.5 MS [M-H$^+$] and [M-H$^-$] spectrum of compound 1



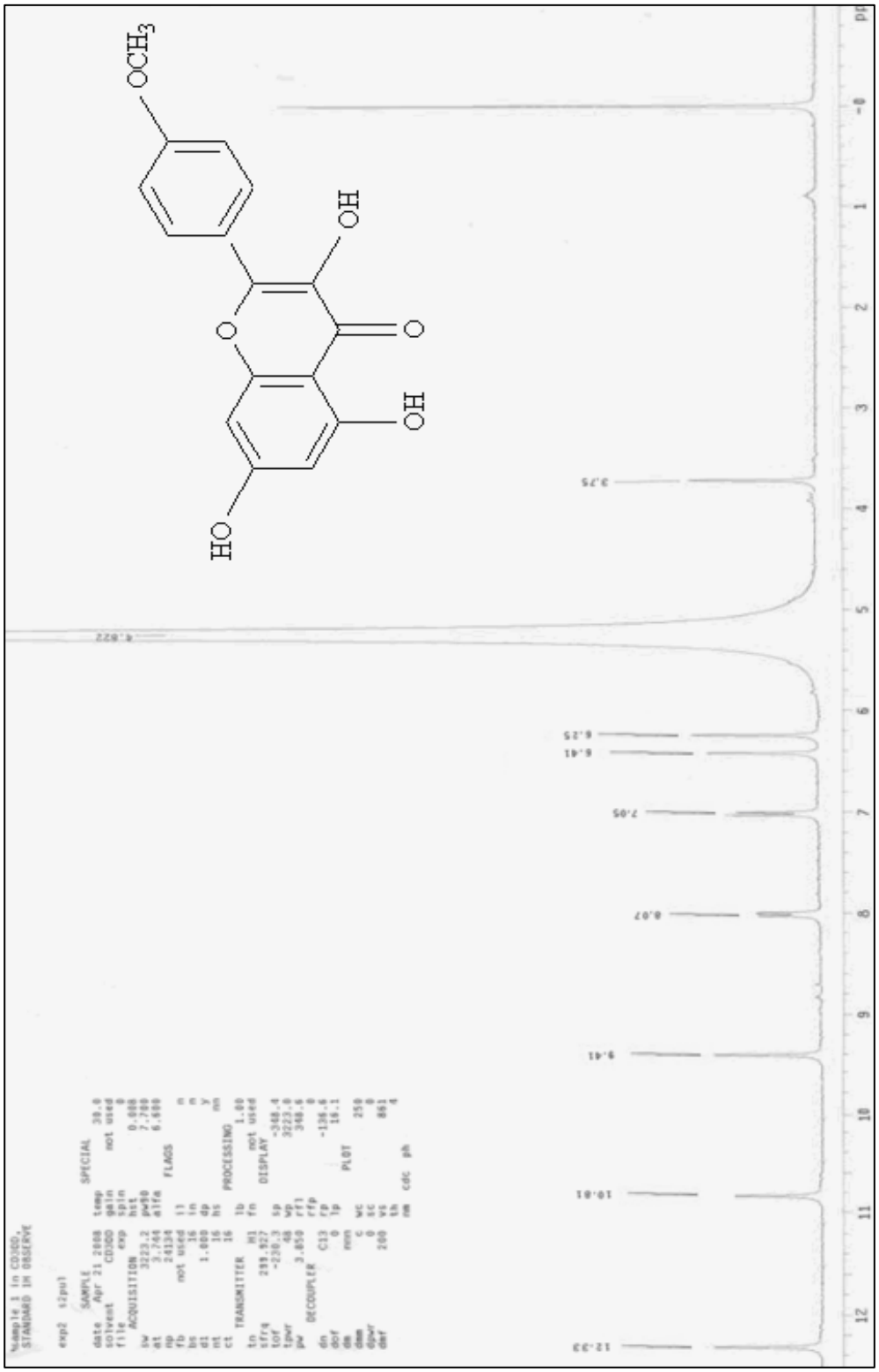
A.6 UV-Vis spectrum of compound 2



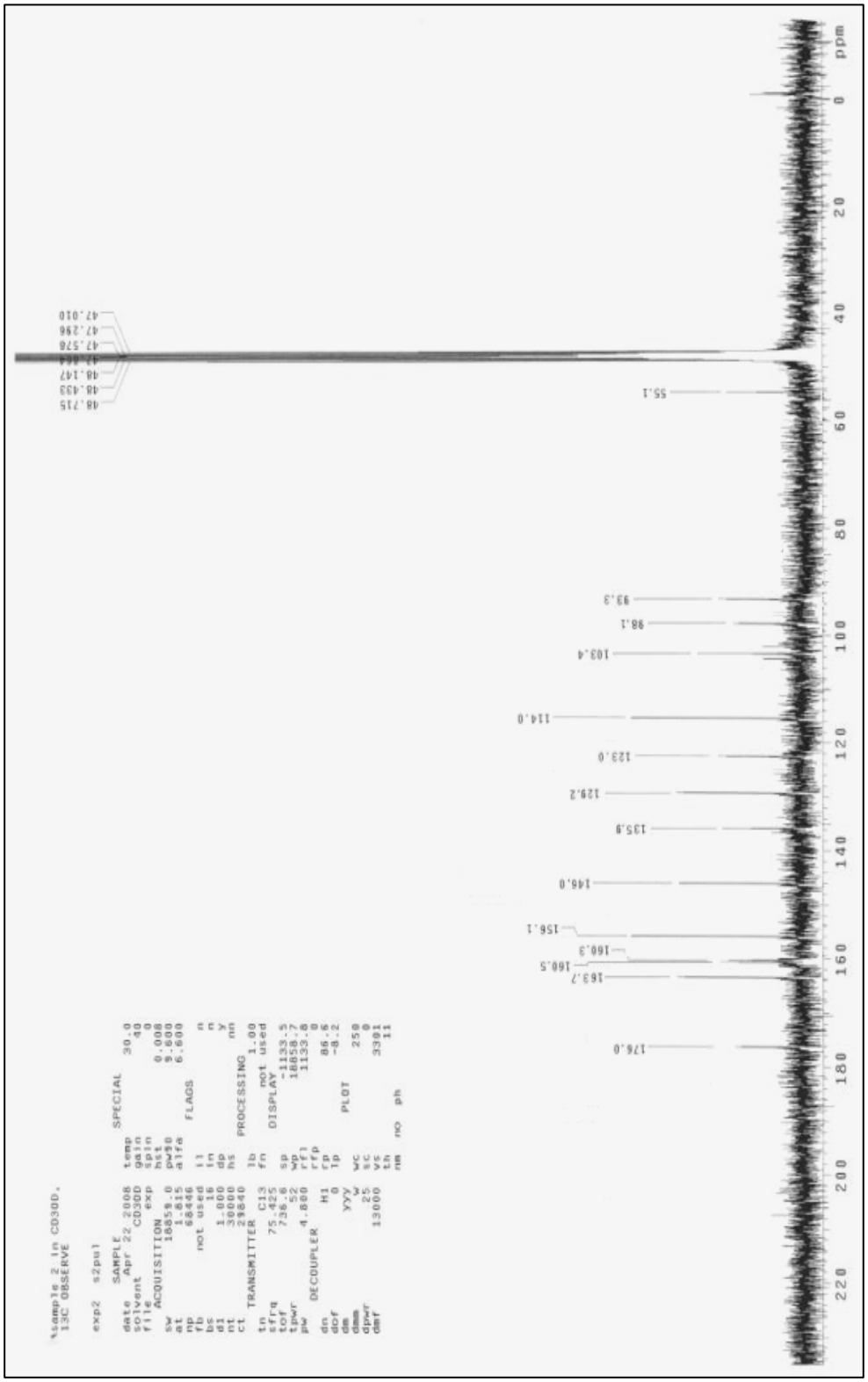
A.7 IR spectrum of compound 2



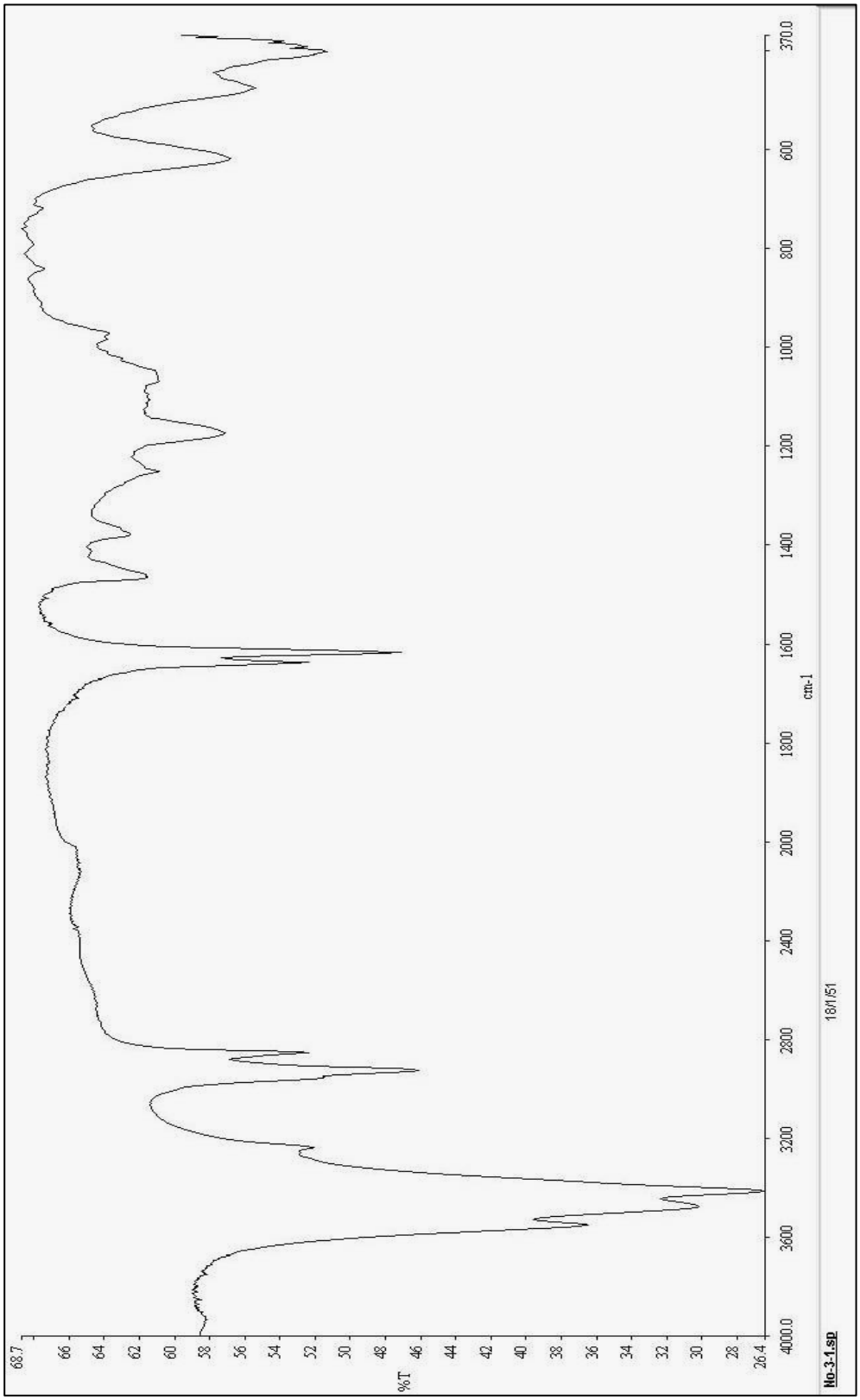
A.8 ^{1}H-NMR spectrum of compound 2



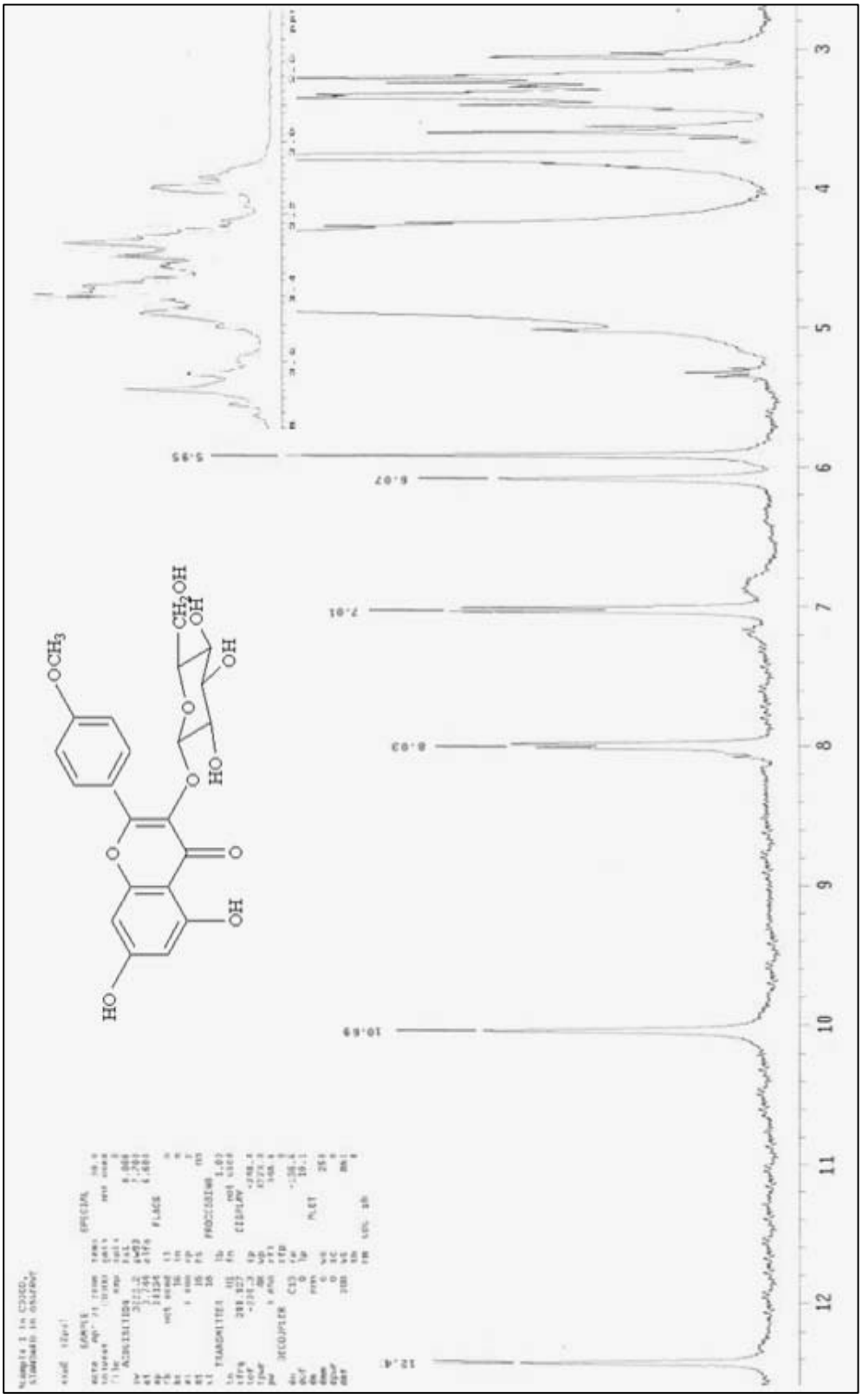
A.9 ^{13}C-NMR spectrum of compound 2



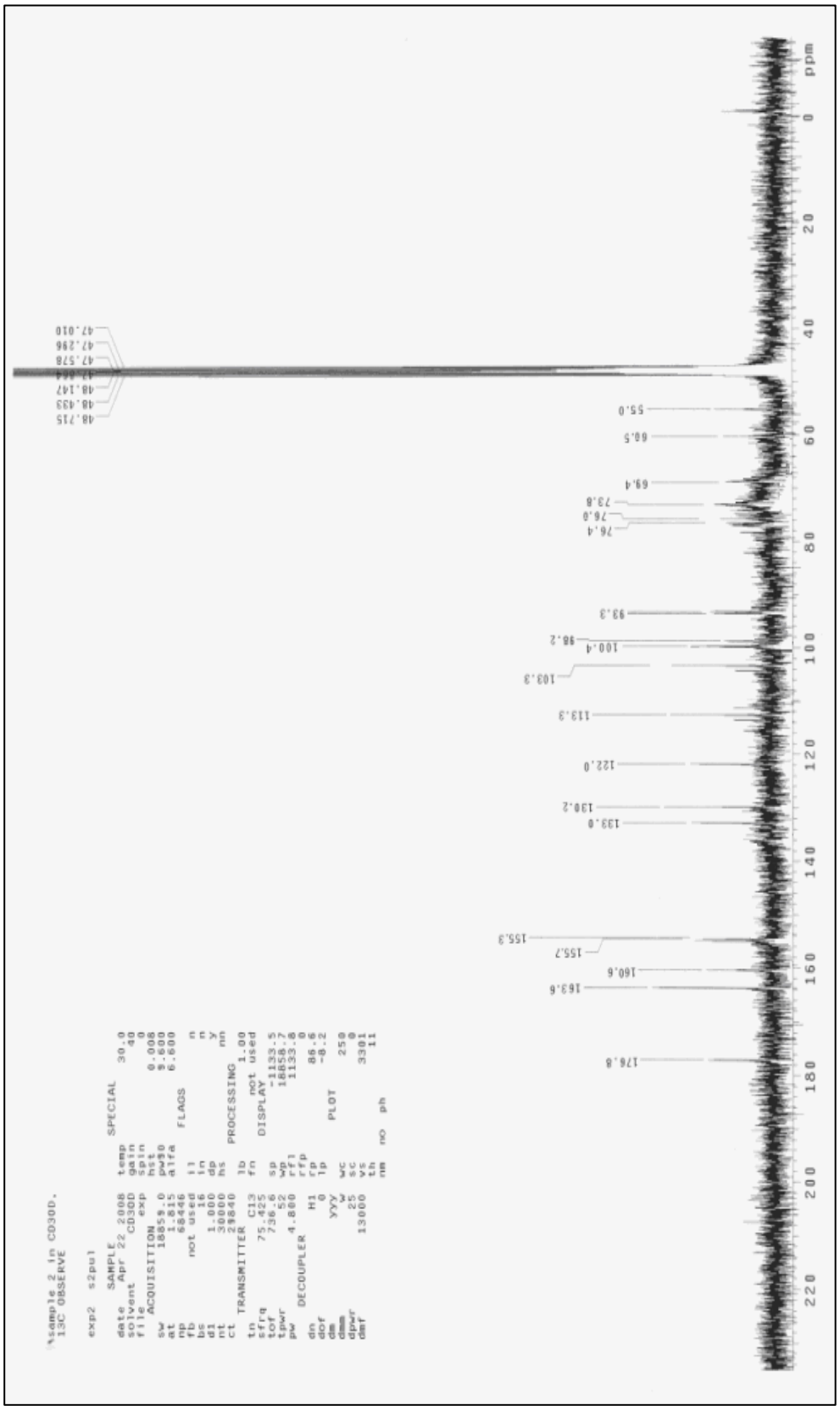
A.10 IR spectrum of compound 3



A.11 ^{1}H-NMR spectrum of compound 3



A12 ^{13}C-NMR spectrum of compound 3



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