สารควบคุมการเติบโตของพืชจากเชื้อรา *Alternarai porri*

นายเสกสรร หล่อสกุลรัตน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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PLANT GROWTH REGULATORS FROM FUNGUS *Alternaria porri*

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เสกสรร หล่อสกุลรัตน์ : สารควบคุมการเติบโตของพืชจากเชื้อรา *Alternaria porri* (PLANT GROWTH REGULATORS FROM FUNGUS *Alternaria porri*) อ. ที่ปรึกษา: ผศ. ดร.สันติ ทิพยางค์, อ. ที่ปรึกษาร่วม: อ. ดร. ปรีชา ภูวไพรศิริศาล, 52 หน้า. ISBN 974-53-1202-9

งานวิจัยนี้มีวัตถุประสงค์เพื่อแยกและจำแนกสารทุติยภูมิที่มีฤทธิ์เป็นสารควบคุมการเจริญเติบโตของพืชจากเชื้อราที่ก่อโรคในพืช จากการคัดเลือกเชื้อราที่ก่อโรคในพืช 5 สายพันธุ์ ได้แก่ *Alternaria porri*, *Trichoderma virens*, *Sclerotium rolfsii*, *Phytophthora parasitica* และ *Trichoderma hazeanium* พบว่า สิ่งสกัดเอทิลอะซิเตตที่สกัดได้จากอาหารเหลวที่เลี้ยงเชื้อรา *A. porri* แสดงฤทธิ์ในการยับยั้งการเติบโตในระยะต้นกล้าของผักกาดขาว (*Brassica pekinensis* Rupr.) ได้ดีที่สุด จากสิ่งสกัดเอทิลอะซิเตตสามารถแยกสารได้ทั้งหมด 5 ชนิดได้แก่ altersolanol A (**1**), altersolanol B (**2**), altersolanol C (**3**) และสารที่ยังไม่ทราบโครงสร้างแน่นอนอีก 2 ชนิด ได้แก่ สารที่ **4** และ **5** การพิสูจน์สูตรโครงสร้างของสารทั้ง 5 โดยอาศัยหลักการของนิวเคลียร์แมกเนติกเรโซแนนซ์ และการเปรียบเทียบกับข้อมูลที่มีในรายงาน

การทดสอบฤทธิ์ทางชีวภาพในการเป็นสารควบคุมการเจริญเติบโตของพืชในระยะต้นกล้ากับผักกาดขาว และหญ้าข้าวนก (*Echinochloa crus-galli* Beauv.) ที่ความเข้มข้น 1,000 ppm พบว่า สารที่มีปริมาณมากที่สุด **1** แสดงฤทธิ์ยับยั้งการเติบโตได้อย่างสมบูรณ์ในผักกาดขาวและหญ้าข้าวนก โดยที่สาร **2** และ **3** แสดงฤทธิ์ยับยั้งการเติบโตใกล้เคียงกับสาร **1** และสารที่แสดงฤทธิ์ต่ำ ได้แก่ สาร **4** และ **5**

สาขาวิชา..เทคโนโลยีชีวภาพ.. ลายมือชื่อนิสิต……

ปีการศึกษา…...2547…….. ลายมือชื่ออาจารย์ที่ปรึกษา……..

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……..……..

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KEY WORD: *Alternaria porri* / CHEMICAL CONSTITUENTS / PLANT GROWTH REGULATORS

SAKESAN LORSAKUNRAT: PLANT GROWTH REGULATORS FROM FUNGUS *Alternaria porri.* THESIS ADVISOR: Assistant Professor Santi Tip-pyang, Ph.D. THESIS CO-ADVISOR: Preecha Phuwapraisirisan, Ph.D., 52 pp. ISBN 974-53-1202-9

The objective of this research was the isolation and identification of plant growth regulators from secondary metabolites of plant pathogenic fungi. Five plant pathogenic fungi (*Alternaria porri*, *Trichoderma virens*, *Sclerotium rolfsii*, *Phytophthora parasitica* and *Trichoderma hazeanium*) were selected for further plant growth regulators screening test. The crude ethyl acetate from the liquid culture of *A. porri* showed the highest inhibitory effect against lettuce seedling (*Brassica pekinensis* Rupr.). This crude extract was isolated to yield 5 compounds. They were altersolanol A (**1**), altersolanol B (**2**), altersolanol C (**3**) and two possible structural compounds (**4** and **5**). The structures of all compounds were determined on the basis of their NMR and also comparison with the literature data.

The biological activity on plant growth regulators of lettuce and *Echinochloa crus-galli* Beauv. seedling, the major component **1** showed completely inhibition of both plants at concentration of 1,000 ppm, while **2** and **3** showed close inhibitory activity to **1**. However, **4** and **5** gave low activity.

Field of study…Biotechnology….. Student's signature……..

Academic year…….2004……... Advisor's signature……..

Co-advisor's signature…….

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The author would like to express his deep gratitude to his beloved parents, dear sister and brother for their great support and encouragement. Finally, he will memorize all of his friends for their friendship and help throughout his studies.

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List of Abbreviations

^{o}C	degree Celsius
^{o}F	degree Fahrenheit
CC, SiO_2	column chromatography using silica gel as absorbent
δ	chemical shift
d	doublet
dd	doublet of doublet
DMSO	dimethyl sulfoxide
Hz	hertz
HPLC	high performance liquid chromatography
J	coupling constant
m	multiplet
mg	milligram
mL	milliliter
nm	nanometer
NMR	nuclear magnetic resonance
ppm	part per million (or mg/L)
PDA	potato dextrose agar
PDB	potato dextrose broth
R_f	retardation factor
R_t	retention time
s	singlet
t	triplet
TLC	thin layer chromatography
UV	ultra-violet
µL	microliter

CHAPTER I

INTRODUCTION

Over the past decades, bioactive metabolites of microbial origin have been the subject of scientific research in several fields, including pharmacology, food science, mycology and plant pathology. A relatively novel and promising field of study is the application of these compounds in agriculture, as pesticides, herbicides or plant growth regulators.

Secondary metabolites produced by microorganisms provide a rich source of novel biological active compounds. However, only a small percentage of the microorganisms has been investigated worldwide phytochemically until now. Therefore, searching for natural substances especially with new mode of actions is still one of the very important aspects with a big potential in the field of fungus toxins. (Fakhouri et al., 2001)

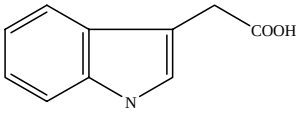
Fungus toxins are the compounds that cause a pathological condition in some host, plant, or animal. This may involve the invasion of the host by the fungus (parasitism), the ingestion of the fungus (mushroom), or the ingestion of products on which the fungus has grown (food poisoning). The studied on the involvement of the plant disease caused by pathogenic microorganism, showed the grouping of their natural toxins, (Griffin, 1994) such as, terpeniod toxins, phenolic toxins, glycoprotein toxins and toxins derived from amino acids.

Many different amino acids were assembled into the molecule of toxins derived from amino acids. Two groups of toxins stand out because of current patterns in their molecular structure, the cyclic peptides and the indole alkaloids. Several toxins derived from amino acids do not have peptide bonds, such as, psilocybin, bufotenine and sporodesmin. The indole alkaloids (psilocybin, bufotenine and ergot toxins) were nerve poisons that cause hallucinatory symptoms, probably because of their chemical similarity to serotonin. There were no known phytotoxics indole compounds, although the plant growth regulator indoleacetic acid synthesized by many fungi many be implicated in plant disease.

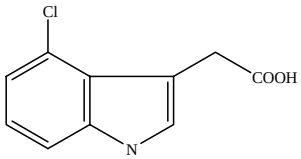
One of well known agrochemicals, plant growth regulators are classified as pesticides, although their functions are very different. Pesticides essentially protect crops and prevent them from blight cause by diseases, insects and other factors. Plant growth regulators are applied on crops to increase yield and improve quality, thereby meeting commercial demand and quality standards. They also regulate the dormancy state of seed and bud, control the development of plant parts or organs, increase resistant tillering and also control blossoming. Other functions of plant growth regulators are to prolong the abscission state, prevent the plant part from falling to the ground, prolong senescent state and ease harvesting. Plant growth regulators are used to control and enhance specific chemical processes in plant to meet the demands of human consumption.

Plant growth regulators usually are defined as organic compounds, other than nutrients, that affect the physiological processes of growth and development implants when applied in low concentrations (Nickell, 1982). For practical purposes, plant growth regulators can be defined as either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting. Plant growth substances (Hopkins, 1999) were classified into five common classes, auxins, gibberellins, cytokinins, ethylene and growth retardants or inhibitors.

Auxins (Stern, 2003), were the first plant hormone to be discovered. There molecules had similar structure to those of the amino acid tryptophan, which was found in both plant and animal cells. The well known natural auxins were indolacetic acid (IAA), phenylacetatic acid (PAA) and 4-chloroindole-3-acetic acid (4-Cl-IAA). They promoted shoot growth, stem growth, cell division in the cambium, differentiation of cell types, cell enlargement and initiation of roots but inhibited root growth and lateral branching, delay developmental processes such as fruit and leaf abscission and fruit ripening. Sensitivity to auxins was less in many monocots than it was in dicots. Low concentration of synthetic auxins which were used as herbicide in horticulture and agriculture (Taiz and Zeiger, 2002), such as naphthalene acetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA), had property of herbicide to kill some broad-leaved plants.

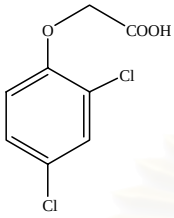


Indolacetic acid

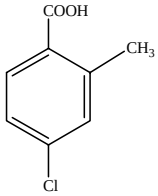


4-chloroindole-3-acetic acid

Natural Auxins



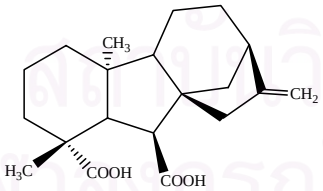
Dichlorophenoxyacetic acid



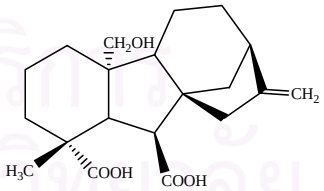
2-methyl-4-chlorophenoxyacetic acid

Synthetic Auxins

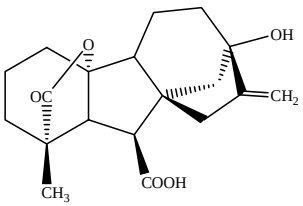
Gibberellins (Stern, 2003) were a large group of related chemical compounds (over 110 have been identified) with wide range of effect. They not only increased stem elongation, especially, induced internode elongation (Taiz and Zeiger, 2002) but they were also involved in nearly all of the same regulatory processes in plant development as auxins. Sometime, they brought plant to flowering, broke the dormancy phase of buds and seeds and promoted fruit set. GA_{12}, GA_{15} and GA_{20} were biologically active gibberellins which were produced by fungi, *Phaeosphaeria sp*.(Kawaide, 1995)



GA_{12}

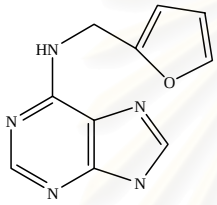


GA_{15}

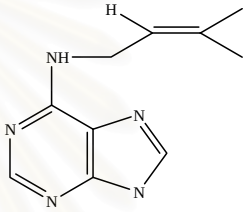


GA_{20}

Cytokinins were groups of chemicals which stimulated plant cell division. The first cytokinin, kinetin was found as a breakdown product of DNA, stimulated the proliferation of parenchyma cell. Many natural cytokinins were discovered, such as, N6-(Δ2-isopentenyl)-adenine (iP), Dihydrozeatin (DZ), both *cis*- and *trans*-zeatin. They also played a role in the enlarging of cell, the differentiation of tissues, the development of chloroplasts, the stimulation of cotyledon growth and prolonged the life of vegetable in storage. Many chemical compounds have been synthesized for antagonized cytokinin, benzyladenine or benzylaminopurine (BA), *N*,*N*'-diphenylurea, 3-methyl-7-(3-methylbutylamino) pyrazolo[4,3-D]pyrimidine and thidiazuron, which was used commercially as a defoliant and an herbicide (Taiz and Zeiger, 2002).

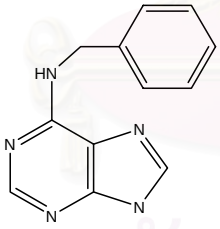


Kinetin

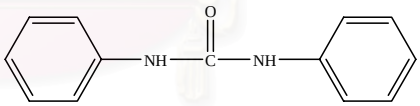


N6-(Δ2-isopentenyl)-adenine

Natural Cytokinins



Benzylaminopurine

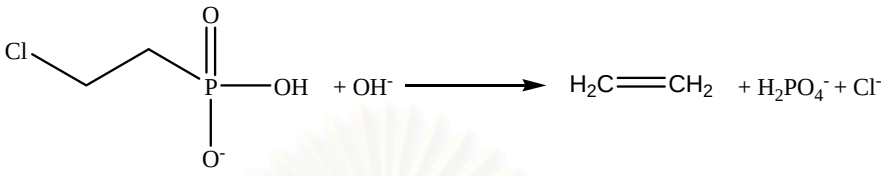


N,*N*'-diphenylurea

Synthetic Cytokinins

Ethylene was a very simple molecule and was produced during many combustion. Ethylene was produced not only by ripening fruits but also by flowers, seeds, leaves and even roots. Environmental stresses and auxins were condition which promoted ethylene production. The ethylene gas (Taiz and Zeiger, 2002) promoted the ripening of fruits, induced lateral cell expansion, induced the formation of roots and root hairs and broke seed and bud dormancy in some plant. In addition, it

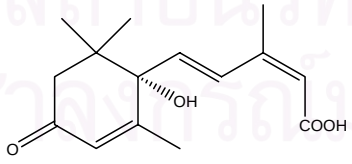
enhanced the rate of leaf senescence. Ethephon (2-chloethylphoshonic acid) was sprayed in aqueous solution and was readily absorbed and transported within the plant. It released ethylene slowly by a chemical reaction, allowing the hormone to exert its effects.

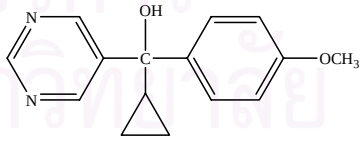


2-Chloroethylphosphonic acid (Ethephon)

Ethylene

Growth retardants (inhibitors) were groups of chemicals, which retarded physiological processes as root and shoot elongation, seed germination, cell division and so on, endogenously regulated plant development and differentiation in cooperation with auxins, cytokinins and gibberellins. Sometime, excretion of retardant from some plant into the environment to stimulated dormancy or inhibited growth of other plant directly or indirectly. One of the most natural active retardant, abscisic acid (Stern, 2003) was synthesized in plastid, apparently carotenoid pigments. It stimulated dormancy in bud and seeds, promoted the formation of abscission layers in leaves and fruits. Many synthetic retardant frequently had a haloaromatic group as partial structure. Ancymidol (Ebrahim, 2004) inhibited gibberellin synthesis which affected stem elongation.





Abscisic acid

Ancymidol

The search for more cost-effective, efficacious, selective and environmentally safe plant growth regulators had led to new emphasis on several underutilized plant growth regulator discovery strategies. These include utilization of natural products

from fungi. Natural products offer a huge number of chemical structures and, since many secondary metabolic compounds had potential as leads for new plant growth regulators.

From the results of preliminary screening test of 5 fungi, *Alternaria porri*, *Trichoderma virens*, *Sclerotium rolfsii*, *Phytophthora parasitica* and *Trichoderma hazeanium*, the ethyl acetate extract from liquid culture of *A. porri* showed the best results of seedling growth activity against *Brassica pekinensis* Rupr. then *A. porri* was selected for further investigation on chemical constituents.

Description and Diseases of *Alternaria porri* (Holliday, 1995)

Characteristic of *A. porri*

Conidiophores: arising singly or in groups, straight or flexuous, sometimes geniculate, septate, pale to mid brown, up to 120 µm long, 5-10 µm thick, with 1 or several well-defind conidial scars.

Conidia: usually solitary, straight or curved, obclavate or with the body of the conidium ellipsoidal, tapering to the beak which is commonly about the same length as the body but may be shorter or longer, pale to mid-golden brown, smooth or minutely verrucose, overall length usually 100-300 µm, 15-20 µm thick in the broadest part, with 8-12 transverse and 0 to several longitudinal or oblique septa, beak flexuous, pale, 2-4 µm thick, tapering.

Disease of *A. porri* (University of Illinois, Department of Crop Sciences, 1990)

Purple blotch is caused by the fungus *Alternaria porri* which commonly follows injury produced by downy mildew and Botrytis leaf blight infections. Besides common onion, the Egyptian onion, Welsh or Spanish onion, garlic, leek, false shallot, and possibly other members of the onion family may become infected. Yellow globe cooking-type onions are less affected than sweet spanish types. The first appears as small, whitish sunken lesions. Almost immediately, the spots turn brown, enlarge, become zoned, somewhat sunken, and more or less purplish (Figure1.1 b). The lesions occur on the leaves, flower stalks, and floral parts of seed onions. When conditions are favorable for disease development, the lesions quickly girdle the leaves and seed stems. Affected leaves and stems may turn yellow, die back, collapse, and die within several weeks after the first lesions appear. In moist weather, diseased tissues are covered with a dense, dark purplish black mold composed of large numbers of microscopic, dark multicelled spores. The conidia are carried to other onion leaves by

air currents, splashing rains, tools, and so on. When the spores land on susceptible onion tissue they germinate in a film of water, and the germ tubes penetrate the stomates or penetrate directly through the epidermis. Early symptoms can appear 1 to 4 days after penetration has occurred. A new generation of conidia may be produced every 5 days in warm, moist weather. Infection, reproduction, and spread of the disease may follow in rapid succession as long as favorable conditions persist. Free moisture, in the form of rain, persistent fog, or dew, is required for infection and spore production. Mycelial growth of the *Alternaria* fungus occurs over a temperature range of 43° to 93°F or 6° to 34°C (optimum 77° to 81°F or 25° to 27°C) at a relative humidity of 90 percent. Almost no infection occurs below 55°F or 24°C). In addition, onion bulbs become infected at harvest or later in storage through the neck or through wounds in the fleshy bulb scales. The rot is first semi-watery and a deep yellow but gradually turns a wine-red, finally becoming dark brown to black. Only one or two outer scales are often affected. Diseased bulb tissue gradually dries out and becomes papery. Sweet spanish onions are very susceptible, while varieties with waxy foliage have some resistance.

Figure 1.1 Infected onion bulbs by *A. porri* (a), Purple blotch lesion on onion leaves (b), *A. porri* growth on liquid culture (c), Purple blotch and necrosis lesion on onion leaves (d), *A. porri* growth on semi-solid culture (e)

1.1 The Chemical Constituent Studies on *Alternaria porri*

Chemical constituents of *Alternaria porri* was initially investigated since 1984. Suemitsu et al. have been reported the isolation of three bianthraquinone red pigments named alterporriol A (**1**), alterporriol B (**2**) and alterporriol C (**3**) as causative toxins of purple blotch disease (Suemitsu et al., 1987 and 1988).

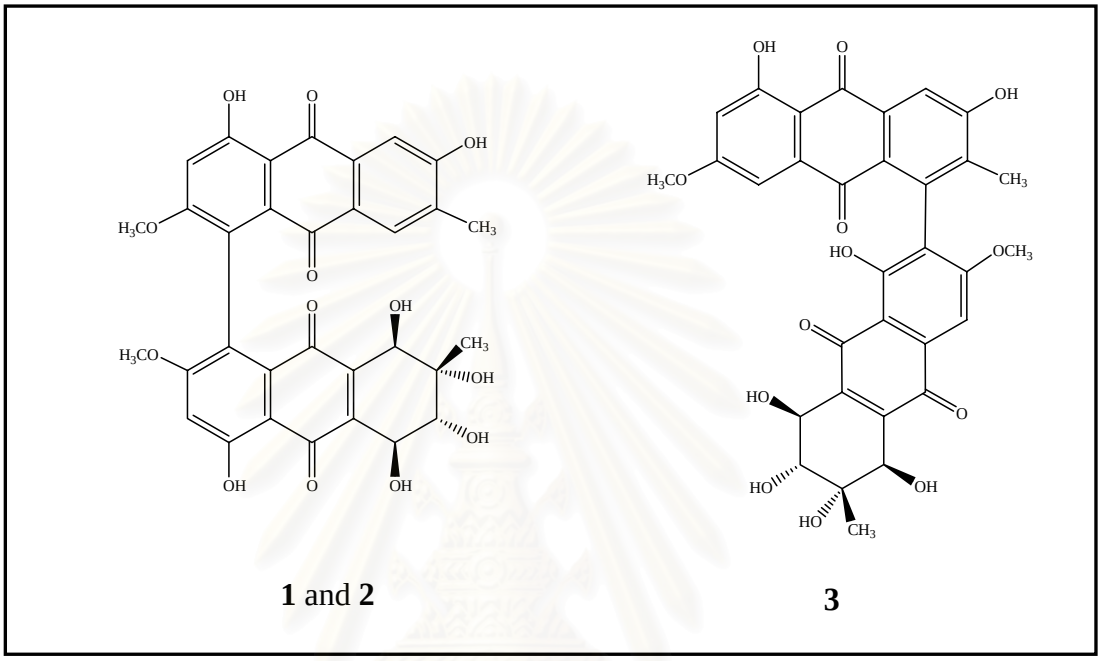
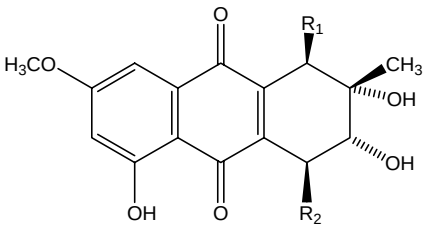
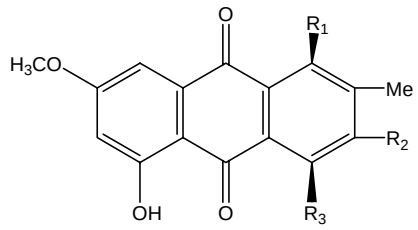


Figure 1.2 Bianthraquinones from *A. porri*, **1** and **2** are atropisomers

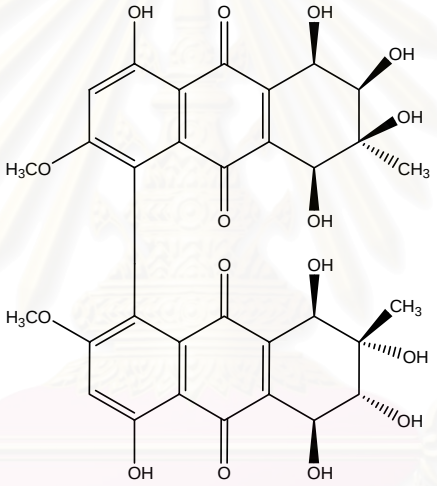
Furthermore, they also found eleven pigments from *A. porri* (Suemitsu et al., 1990). Five pigments were bianthraquinone, **1**, **2**, **3**, alterporriol D (**4**) and alterporriol E (**5**). Six pigments were anthraquinone derivatives, namely macrosporin (**6**), 3-methylxathopurpurin-6-methylether (**7**), 1,2,8-trihydroxy-6-methoxy-3-methylanthraquinone (**8**), altersolanol A (**9**), altersolanol B (**10**) and altersolanol C (**11**). These bianthraquinone **4**, **5** and anthraquinone **6**-**11** also were found to be pigments in *Alternaria solani*. The relationship between **1** and **2**, **4** and **5** are found to be atropisomers of each other. In addition, **4** and **5** were investigated for their biosynthesis pathway. Their incorporation experiments of single and double labeled acetates suggested that both **4** and **5** were presumably derived from oxidative coupling of **9** (Ohnishi et al., 1991).



	R_1	R_2
9	OH	OH
10	H	H
11	OH	H



	R_1	R_2	R_3
6	H	OH	H
7	H	H	H
8	H	OH	OH



4 and **5**

Figure 1.3 anthraquinones and bianthraquinones pigments from *A. porri*, **4** and **5** atropisomer.

In 1992, Suemitsu et al. reported the other types of phytotoxins. In addition, aforementioned anthaquinones Zinniol (**12**) and porritoxin (**13**) were isolated, in which the latter highly inhibited seedling growth at concentrations as low as 10 ppm. The structure of porritoxin was later revised to posses isoindole moiety instead of benzoxazocine derivative (Horiuchi et al., 2002).

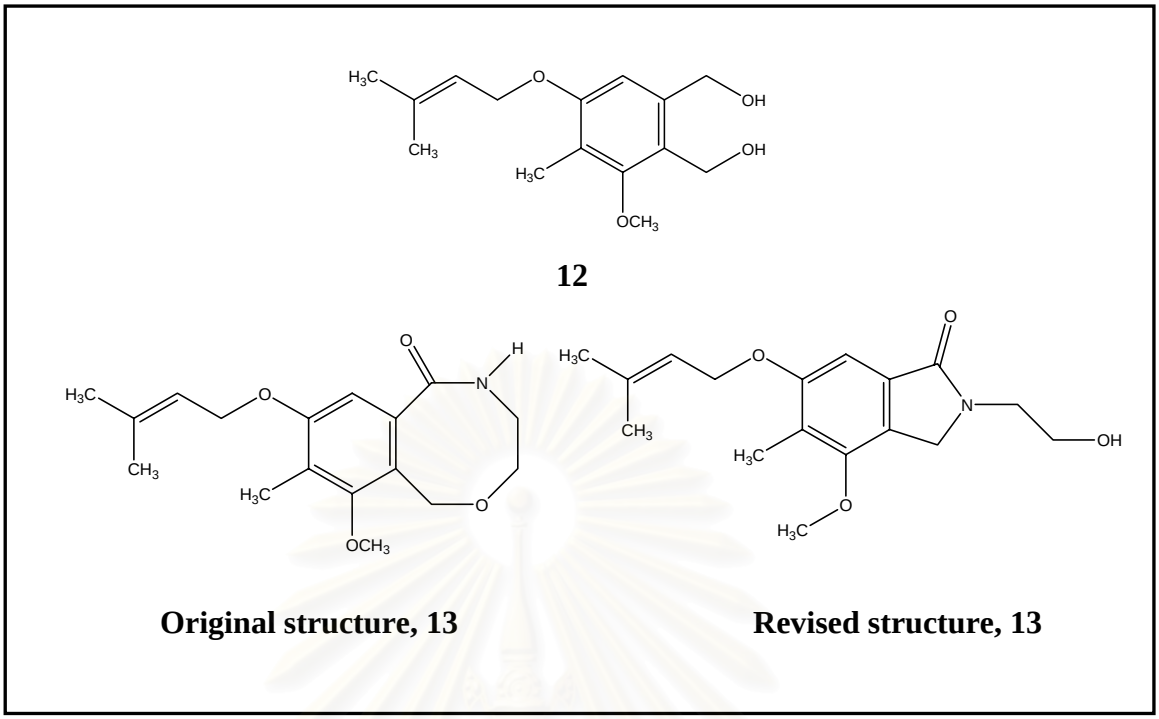


Figure 1.4 The structures of metabolites from *A. porri*

The same group subsequently isolated two phytotoxins (Suemitsu et al., 1994), porriolide (previously called silvaticol (**14**)) and porritoxinol (**15**), which suppressed the growth of lettuce seedling at concentration of 12.5 ppm.

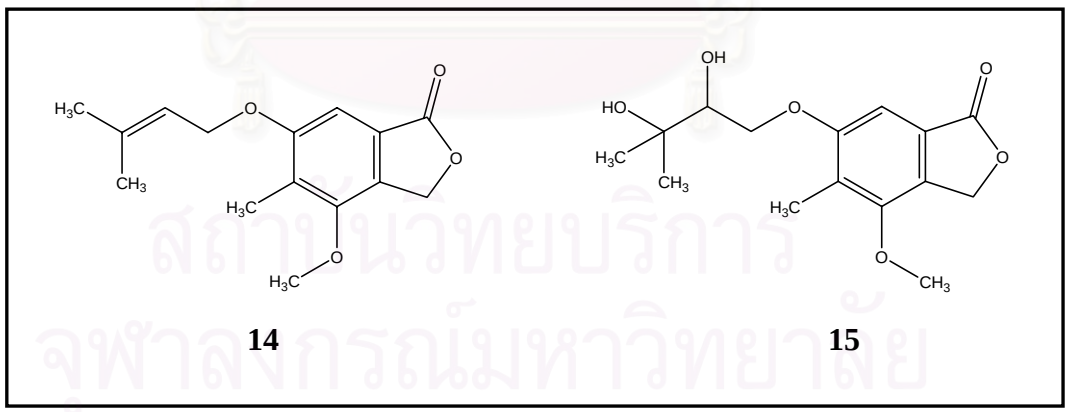


Figure1.5 The structures of metabolites from *A. porri* (cont.1)

Two phytotoxins (Suemitsu et al., 1995), zinnimidine (**16**) and 5-(3′,3′-dimethylallyloxy)-7-methoxy-6-methylphthalide (**17**) were investigated by the same group. The phytotoxic activity was not observed.

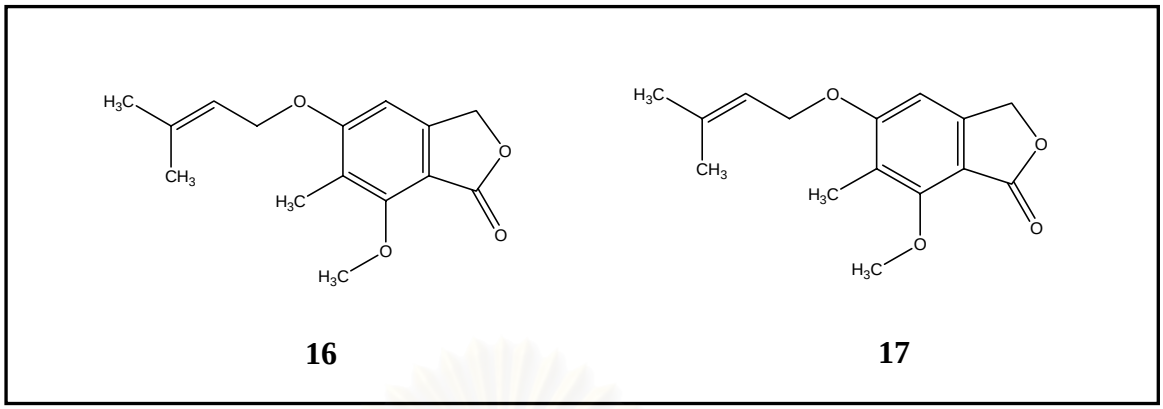


Figure 1.6 The structures of metabolites from *A. porri* (cont.2)

The final chemical study was terminated by the investigation of Horiuchi *et al*. They found porritoxin like-structure, porritoxin sulfonic acid (**18**), and their phytotoxicity. The repressed growth of **18** on lettuce and stone-leek seedling was investigated.

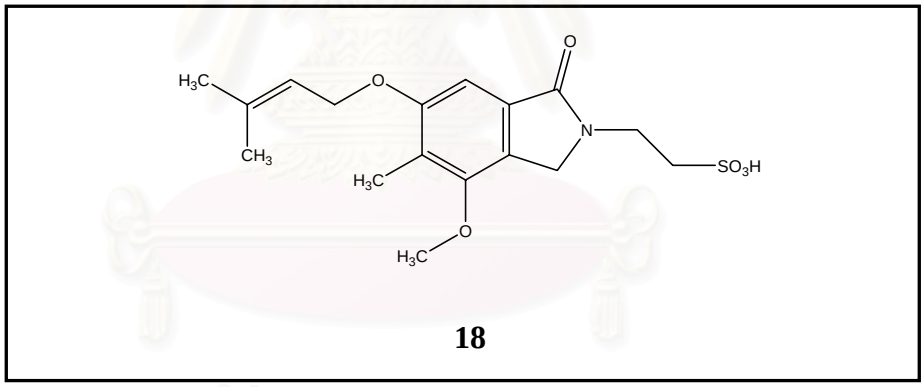


Figure 1.7 The structures of metabolites from *A. porri* (cont. 3)

The structural activity relationship of three isoindolines, **13**, **16** and **18**, was investigated for inhibitory effect on seedling. The compound 1**3** showed the strongest activity while compound **18** exhibited the weakest activity. The primary hydroxyl group of **13** showed the strongest effect among them. The *N*-alkyl and hydroxyl groups seem to be contributed to the activity when compare with **16**. In addition, activity was dropped when sulfonyl group of **18** was replaced with hydroxyl group.

1.2 The Goal of this Research

Based on primary screening results on seedling growth of *Brassica pekinensis* Rupr. (Lettuce) of plant pathogenic fungi. *Alternaria porri* showed the potent activity, was selected for further investigation on chemical constituents and plant growth regulator activity. The goal of this research could be summarized as follows:

1. To extract and isolate the organic substances from the liquid culture of *Alternaria porri*.
2. To elucidate the structural formula of the isolated substances from the active crude extract which show the promising activity.
3. To evaluate the plant growth regulator activity of isolated compounds against *B. pekinensis* Rupr. and *Echinochloa crus-gali* (L.) Beauv.

The separation and structure elucidation of all compounds will be carried out by chromatographic and spectroscopic techniques.

CHAPTER II

SCREENING FOR PLANT GROWTH REGULATORS FROM PLANT PATHOGENIC FUNGI

2.1 Screening Results

The screening results of ethyl acetate extracts from liquid culture of 5 fungi were presented in Table 2.1

Table 2.1 Effect of ethyl acetate extracts of liquid culture of plant pathogenic fungi on seedling growth of *B. pekinensis* Rupr.

Fungi	Level of inhibition at concentration (ppm)		
	10	**100**	**1,000**
Alternaria porri	++	+++	+++++
Trichoderma virens	+	++	+++
Sclerotium rolfsii	+	+++	++++
Phytophthora parasitica	+	++	+++++
Trichoderma hazeanium	+	+	++

* The level of plant growth inhibition was determined by using the approximation of root and shoot lengths compared with the control; thus, the level was classified as inhibition more than 90% (+++++), 60-90% (++++), 40-60% (+++), 20-40% (++) and less than 20% (+).

Based on the result of *B. pekinensis* Rupr. seedling growth inhibitory effect, ethyl acetate extracts of *A. porri* , *S. rolfsii* and *P. parasitica* showed complete inhibition at 1,000 ppm. Thus, extract yield and TLC pattern were criteria to select candidate fungi. *A. porri* apparently produced extract yield (0.9 g/L) more than those produced by both *S. rolfsii* (0.19 g/L) and *P. parasitica* (0.4 g/L), while its TLC pattern was strikingly interesting. Thus, *A. porri* was selected for further investigation.

2.2 Plant Growth Regulation Assay

Plant growth regulator test was used as a main bioassay to verify the presence of bioactive compounds. Selected plants for this investigation were *Brassica pekinensis* Rupr. (lettuce) (Takeuchi et al.,1995) and *Echinochloa crus-gali* (L.) Beauv. (Kawaguchi et al., 1997). The bioassay was performed as follows:

Test solutions were prepared at concentrations of 1,000, 100 and 10 ppm. One hundred microliters of test solutions was added into glass vial (2 cm i.d.) for *B. pekinensis* Rupr. that contained a filter paper disc for *B. pekinensis* Rupr. A control was an equal amount of the solvent used to prepare the test solution. All glass vials were dried. Five seedlings were placed in a glass vial wetted with 100 µL of distilled water for *B. pekinensis* Rupr. The glass vials were covered with the lid and sealed with parafilm to prevent the evaporation of water. These glass vials kept under daylight for three days. Growth was quantified by measuring the length of roots and shoots. All tests and controls were run in three replicate and averaged. Percent of growth inhibition could be calculated from the following formula. In case of *E. crus-gali* (L.) Beauv., the test solution was added into glass vial for 500 µL and wetted with 500 µL of distilled water.

$$\%\text{Growth Inhibition} = \left[1 - \left(\frac{T}{C}\right)\right] \times 100\ \%$$

When "T" was the mean roots or shoots length of treated seedling

"C" was the mean roots or shoots length of controlled seedling

Growth inhibition of 100 % revealed a complete inhibitory effect

2.3 Fungal Materials and Fermentation

2.3.1 Fungal Materials

The plant pathogenic fungi used in this experiment were provided by Department of Plant Pathology, Faculty of Agriculture, Kasetsart University (*Alternaria porri*) and Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand (*Trichoderma virens*, *Sclerotium rolfsii*, *Phytophthora parasitica* and *Trichoderma hazeanium*).

2.3.2 Fermentation

Stock culture was activated in semi-solid media, potato dextrose agar (PDA) that was prepared by dissolving dextrose (20 g) and agar (16 g) in 1 L of a potato extract (200 g) solution. The medium (30 mL) in a Petri dish (9 cm in diameter) was inoculated the fungus at room temperature. After 3 days, the cultured agar was punched by 8 mm diameter cork borer and inoculated into 150 mL of liquid media, potato dextrose broth (PDB), which was prepared by dissolving dextrose (20 g) in 1 L of potato extract (200 g) solution. The cultured flask was statically kept at room temperature for 30 days.

2.3.3 Sample Preparation for Preliminary Screening

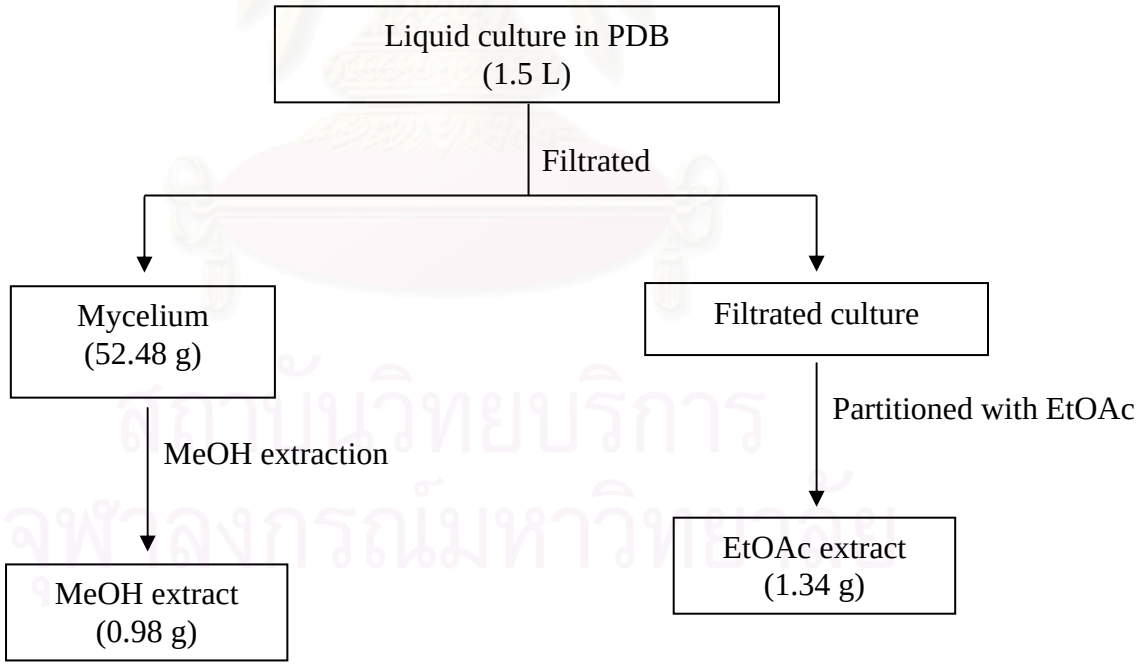
The culture of plant pathogenic fungi was harvested and filtrated through filter paper. The filtrated culture was twice extracted with ethyl acetate. The ethyl acetate layer was dried with anhydrous Na_2SO_4 and then evaporated to dryness for preliminary study.

CHAPTER III

ISOLATION AND STRUCTURE ELUCIDATION OF PLANT GROWTH REGULATOR COMPOUNDS FROM *A. porri*

3.1 Extraction and Isolation

The filtrate (1.5 L) was harvested and filtrated through filter paper. The filtrate was successively extracted with ethyl acetate. The ethyl acetate layer was dried with anhydrous Na_2SO_4, filtrated and evaporated to afford 1.34 g of crude extract. The mycelium was extracted with MeOH to yield 0.98 g of crude extract. The extraction procedure and results for preliminary study are epitomized in Scheme 3.1



Scheme 3.1 Extraction of liquid culture of *A. porri*

3.2 Separation and Purification

Ethyl acetate extract was first separated by vacuum column chromatography. The crude extract was dissolved in little amount of suitable solvent and mixed with silica gel (1:1) to afford the extract paste. The paste was evaporated to dryness by rotary evaporator prior to subject on the top of column. The column was eluted by increasing polarity of solvent. Each fraction was monitored by TLC, and fractions which had the same components were combined, yielding 6 fractions (Table 3.1)

Table 3.1 Separation of ethyl acetate extract by vacuum column chromatography

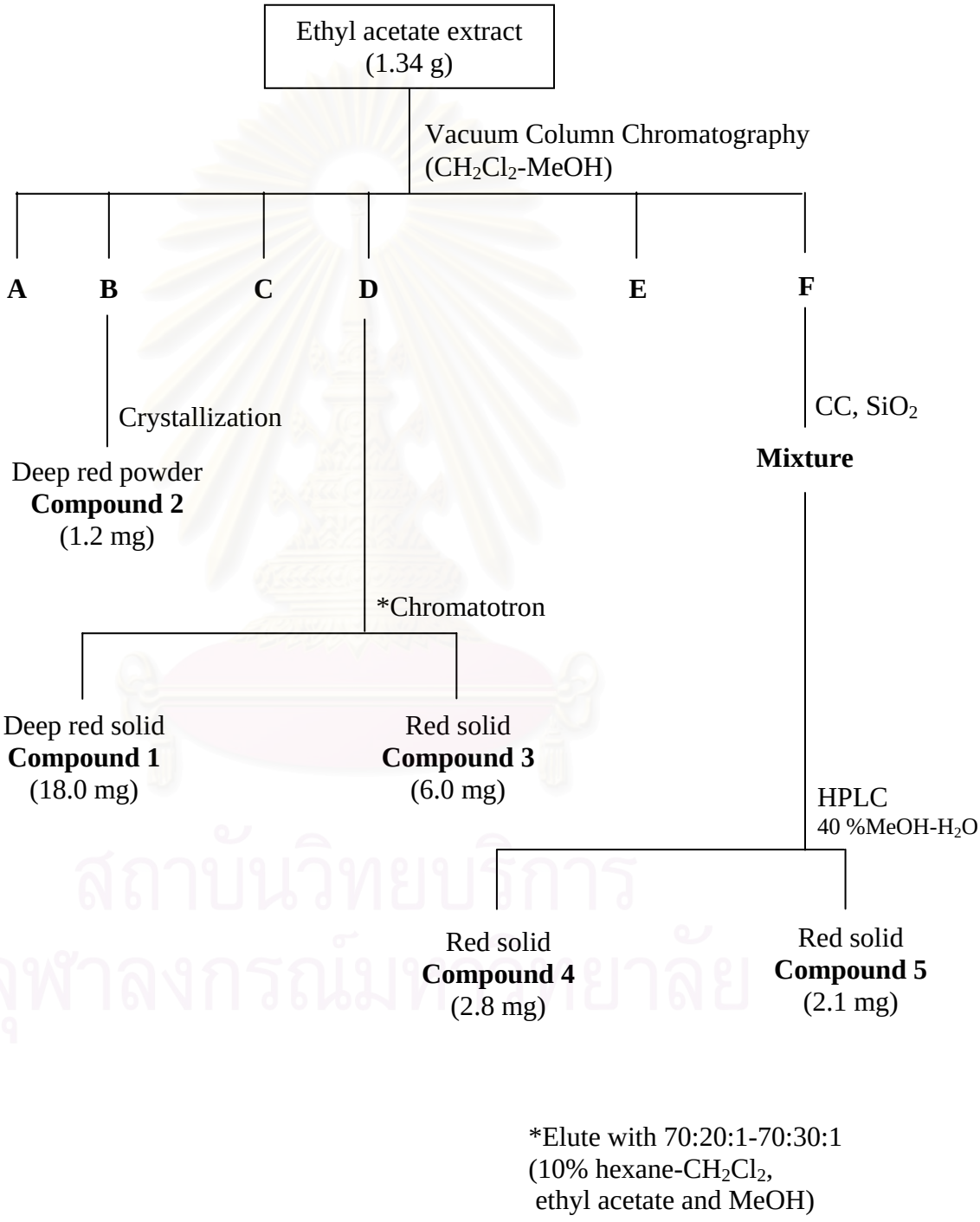
Fraction code	**Solvent system**	**Weight** (mg)
A	CH_2Cl_2 – 1:4 EtOAc/CH_2Cl_2	5.4
B	3:7 EtOAc/CH_2Cl_2	8.2
C	2:3 EtOAc/CH_2Cl_2	15.6
D	1:1-3:2 EtOAc/CH_2Cl_2	36.9
E	7:3-9:1 EtOAc/CH_2Cl_2	6.3
F	EtOAc-MeOH	27.5

After the concentrated fraction B was left overnight, the deep red powder was deposited. It was recrystallized in hot ethyl acetate for several times to yield **compound 2** (0.8 mg/L of liquid culture). From TLC patterns, fraction D showed fluorescence spots under UV light (long wavelength, 365 nm), then chromatotron was used advantageously for the reseparation of this fraction. Elution with 10% hexane-CH_2Cl_2; 70:10:1 - 70:20:1 of 10% hexane-CH_2Cl_2, ethyl acetate and MeOH yielded **compound 3** (4.0 mg/L of liquid culture) and 10% hexane-CH_2Cl_2, 70:10:1 - 70:30:1 of 10% hexane-CH_2Cl_2, ethyl acetate and MeOH yielded **compound 1** (12.0 mg/L of liquid culture).

Fraction F was subjected to silica gel column chromatography eluted with 40% EtOAc-CH_2Cl_2, 10% hexane-EtOAc and 50% MeOH-EtOAc. The last fraction

was further purified by HPLC (C18 reversed-phase, 40% MeOH-H_2O) to yield compound **4** (1.9 mg/L of liquid culture) and **5** (1.4 mg/L of liquid culture) with retention times of 5.1 and 8.1 min, respectively.

The isolation of active compounds from ethyl acetate extract of *A. porri* was briefly summarized in Scheme 3.2



Scheme 3.2 Isolation procedure of the ethyl acetate extract

3.3 Structure Elucidation

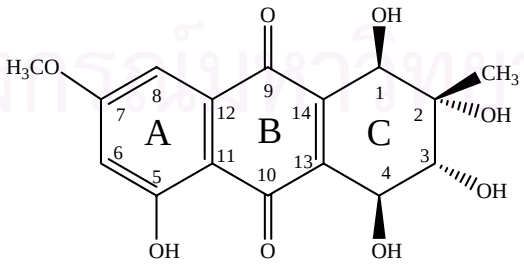
3.3.1 Compound 1

Compound **1** was separated from fraction D of ethyl acetate extract. It was subjected to chromatotron. Elution was performed with 70:30:1 of 10% hexane-CH_2Cl_2, ethyl acetate and MeOH to afford compound **1** (18 mg) as deep red solid with R_f 0.1 (SiO_2 1:3 EtOAc/CH_2Cl_2).

The ^{1}H NMR (DMSO-d_6) spectrum of compound **1** showed presence of five hydroxy protons at δ12.18 (1H, brs), 5.72 (1H, d, *J* = 6 Hz), 5.07 (1H, d, *J* = 5.2 Hz), 4.93 (1H, d, *J* = 6.4 Hz) and 4.50 (1H, overlap), aromatic signals at δ 7.04 (1H, brs) and 6.84 (1H, brs), methine protons at δ 4.50 (1H, overlap), 4.34 (1H, d, *J* = 4.8 Hz) and 3.66 (1H, t, *J* = 6.4, 6.8 Hz), methoxy group at δ 3.87 (3H, s) and methyl group at δ 1.20 (3H, s).

The ^{13}C NMR spectrum of compound **1** demonstrated 16 signals, which were assigned as 2×CH_3, 5×CH and 9×C. A pair of lowfield signals at δ_c 184.2 and 189.0 coupled with aromatic resonance at 163.7, 106.4, 166.0, 107.2, 110.0, 133.8, 145.1 and 142.6 suggested the presence of naphthaquinone moiety. In addition, the remaining oxygenated carbons (δ_c 69.0, 73.4, 74.3 and 69.0) could be assembled to naphthaquinone portion to afford the gross structure of anthraquinone.

According to the above results, NMR data especially ^{13}C NMR (Table 3.2) of compound **1** matched well with those of altersolanol A and comparison of ^{13}C NMR of compound **1** with literature data indicated that compound **1** was altersolanol A.



Compound **1**: altersolanol A

Table 3.2 ^{13}C NMR of compound **1** and altersolanol A in DMSO-d_6 (Stoessl and Unwin, 1979)

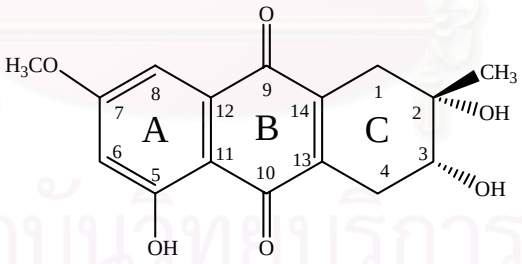
Carbon	Chemical shift (ppm)	
	Compound **1**	altersolanol A
1	69.0	68.6
2	73.4	73.0
3	74.3	73.9
4	69.0	68.7
5	163.7	163.3
6	106.4	105.9
7	166.0	165.6
8	107.2	106.7
9	184.2	183.7
10	189.0	188.6
11	110.0	109.6
12	133.8	133.3
13	145.1	144.5
14	142.6	142.2
2-Me	22.9	22.4
7-OMe	56.8	56.3

3.3.2 Compound 2

Compound **2** was obtained from fraction A of ethyl acetate extract. After crystallization in hot ethyl acetate, compound **2** was formed as deep red powder (1.2 mg) with R_f 0.39 (SiO_2 1:3 EtOAc/CH_2Cl_2).

The ^{1}H NMR (DMSO-d_6) spectrum of compound **2** was closely related to that of altersolanol A (Yagi et al., 1993), except for the presence of two methylene groups at 2.51-2.67 (4H). Compound **2** displayed only two hydroxy protons on ring C at δ 4.44 (OH-2) and 4.77 (OH-3). Therefore, the signals corresponding to the methine protons at C-1 and C-4 of altersolanol A (**1**) were replaced by methylene protons in compound **2**.

According to the above data analysis, compound **2** turned out to be altersolanol B. ^{1}H NMR assignment of **2** compared with previous reported was summarized in Table 3.3.



Compound **2**: altersolanol B

Table 3.3 ^{1}H NMR data of compound **2** and altersolanol B in DMSO-d_6 (Yagi et al., 1993)

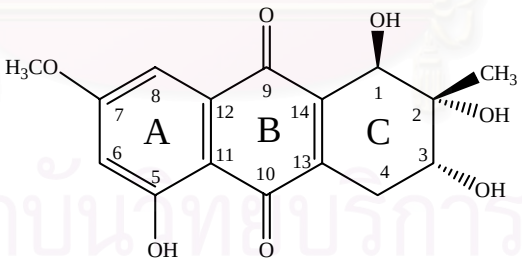
Carbon	Proton	Chemical shift (multiplicity, *J* in Hz)	
		Compound **2**	altersolanol B
1	CH_2	2.51-2.67 (m)	2.55-2.70 (m)
2	OH	4.44 (s)	4.49 (brs)
	CH_3	1.12 (s)	1.19 (s)
3	CH	3.52 (m)	3.55 (m)
	OH	4.77 (d, 5.5)	4.76 (t, 5)
4	CH_2	2.51-2.67 (m)	2.55-2.70 (m)
5	OH	12.21 (brs)	12.31 (brs)
6	CH	6.76 (d, 2.7)	6.77 (d, 2)
7	OCH_3	3.85 (s)	3.88 (s)
8	CH	7.00 (d, 2.7)	6.97(d, 2)

3.3.3 Compound 3

Compound **3** was separated from fraction D of ethyl acetate crude extract. It was subject to chromatotron. Elution was performed with 70:20:1 of 10% hexane-CH_2Cl_2, ethyl acetate and MeOH. Compound **3** (6 mg) was obtained as red solid with R_f 0.18 (SiO_2 1:3 EtOAc/CH_2Cl_2).

The ^{1}H NMR (DMSO-d_6) spectrum of compound **3** was closely related to that of altersolanol A (Yagi et al., 1993), except for the presence of one methylene group at 2.76 and 2.31 (2H). Compound **3** displayed three hydroxy protons on ring C at δ 4.30 (OH-3), 4.38 (OH-2) and 5.50 (OH-1). Therefore, the signal corresponding to the methine proton at C-4 of altersolanol A (**1**) was replaced by methylene proton in compound **3**.

According to the above data analysis, compound **3** turned out to be altersolanol C. ^{1}H NMR assignment of **3** compared with previous report was summarized in Table 3.4.



Compound **3**: altersolanol C

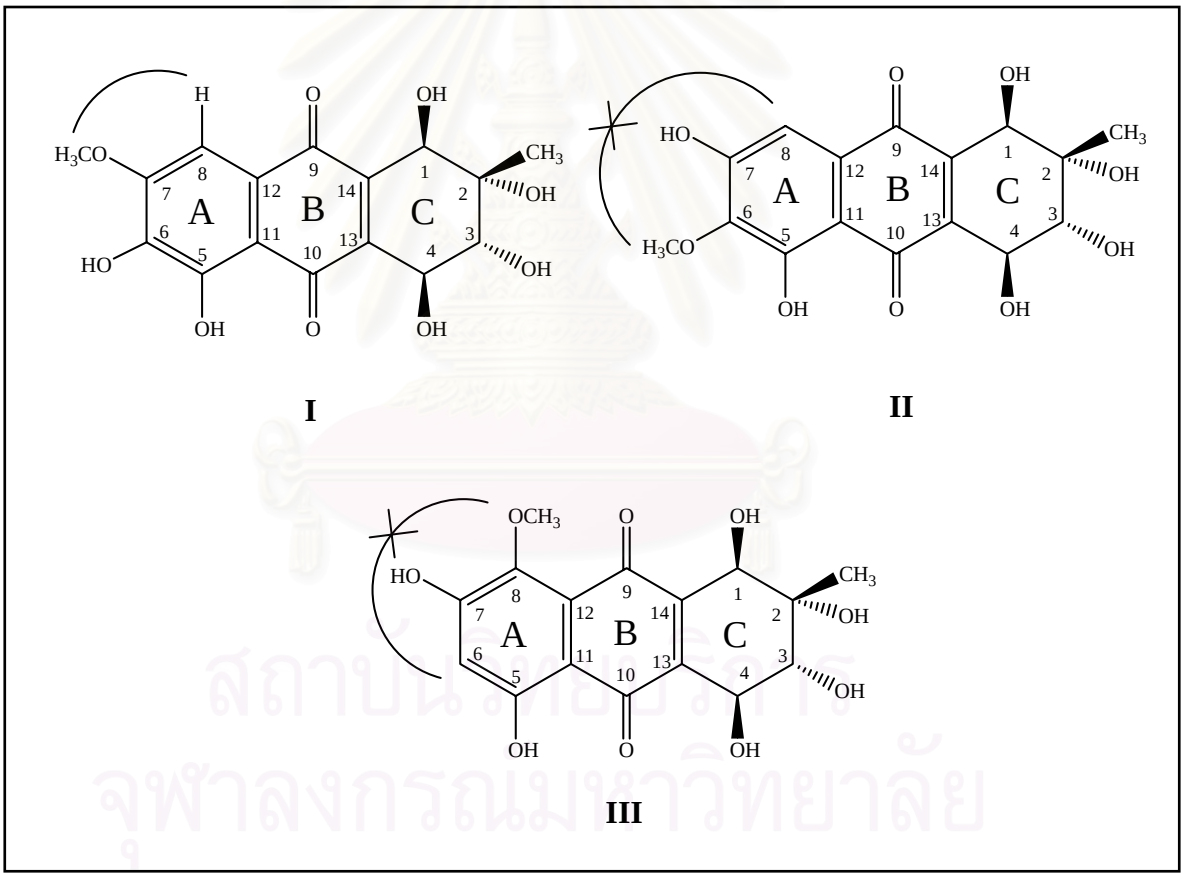
Table 3.4 ^{1}H NMR data of compound **3** and altersolanol C in DMSO-d_6 (Yagi et al., 1993)

Carbon	Proton	Chemical shift (multiplicity, *J* in Hz)	
		Compound **3**	altersolanol C
1	CH	4.67 (d, 6.9)	4.38 (brs)
	OH	5.50 (d, 6.9)	5.51 (s)
2	OH	4.38 (s)	4.32 (brs)
	CH_3	1.23 (s)	1.27 (s)
3	CH	3.70 (m)	3.80 (m)
	OH	4.30 (d, 6.2)	4.56 (dd, 9, 6)
4	CH	2.76 (dd, 18.1, 6.5)	2.30 (dd, 20, 9)
	CH	2.31 (dd, 19.2, 9.6)	2.80 (dd, 20, 9)
5	OH	12.15 (brs)	12.20 (brs)
6	CH	6.78 (d, 2.1)	6.80 (d, 2)
7	OCH_3	3.87 (s)	3.90 (s)
8	CH	7.00 (d, 2.1)	7.02 (d, 2)

3.3.4 Compound 4 and 5

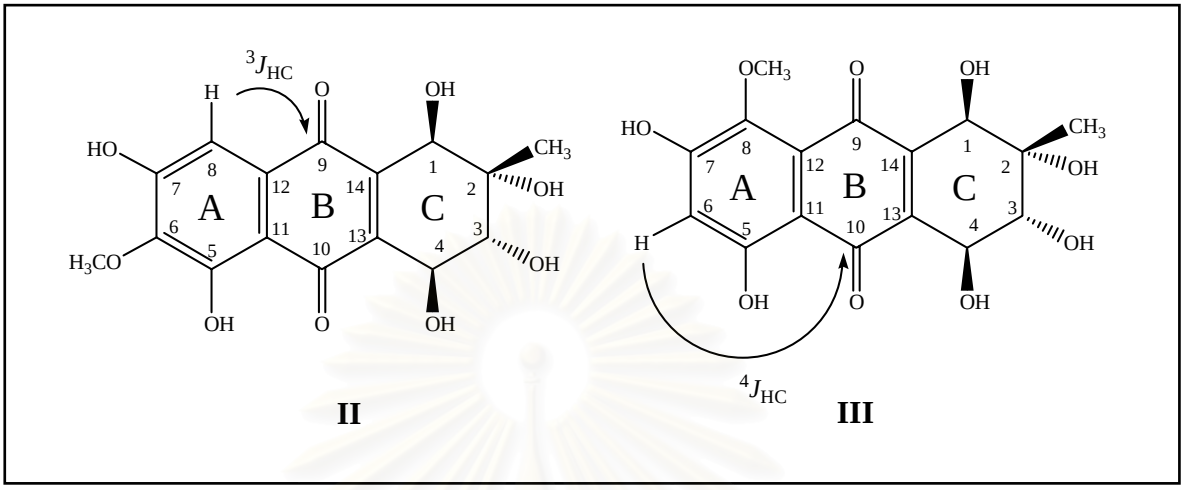
Compounds **4** (2.8 mg) and **5** (2.1 mg) were obtained as deep red solid with identical R_f value from fraction F of ethyl acetate extract. They were separated by column chromatography (silica gel, 10% MeOH-CH_2Cl_2) followed by RP-HPLC (40% MeOH-H_2O) with retention times of 5.1 and 8.1 min.

The ^{1}H NMR (DMSO-d_6) spectra of **4** and **5** were closely related to that of compound **1**, except for the presence of single aromatic proton (δ 6.95 for **4** and 6.93 for **5**) in ring A. Because polarity of **4** and **5** was higher than that of **1**, the ring A should be substituted by hydroxy group. At this moment, there were three possible structures for **4** and **5** (structures **I**-**III**).



The NOE (DMSO-d_6) spectra of compound **4** and **5** showed negative when irradiated aromatic protons at 6.95 and 6.93. In addition, irradiation of methoxy proton at 3.71 and 3.69 showed negative NOE. The negative NOE both aromatic and methoxy proton of **4** and **5** suggested that possible structure should be **II** and **III**.

Theoretically, structures **II** and **III** were possibly differentiated by typical long range coupling of aromatic proton to carbonyl carbon, $^{3}J_{HC}$.



An attempt to obtain such conclusive results from HMBC was not successful due to the limited amount of samples. Therefore, compound **4** and **5** were either **II** or **III**.

Table 3.5 ^{1}H NMR data of compound **4** and **5** compared with compound **1** in DMSO-d_6

Carbon	Proton	Chemical shift (multiplicity, *J* in Hz)		
		Compound **4**	Compound **5**	Compound **1**
1	CH	4.08 (d, 5.1)	4.13 (m)	4.34 (d,4.8)
	OH	5.12 (d, 3.9)	5.08 (d, 6)	5.07 (d,5.2)
2	OH	4.48 (m, overlap)	4.48 (m, overlap)	4.50 (brs, overlap)
	CH_3	1.15 (s)	1.16 (s)	1.26 (s)
3	CH	3.57 (t, 5.1, 5.4)	3.60 (t, 6.4, 7.2)	3.66 (t, 6.4, 6.8)
	OH	4.91 (d, 5.1)	4.89 (d, 6.4)	4.93 (d, 6.4)
4	CH	4.48 (m, overlap)	4.48 (m, overlap)	4.50 (brs, overlap)
	OH	5.69 (d, 5.1)	5.70 (d, 6.8)	5.72 (d, 6)
5	OH	13.11 (brs)	12.96 (brs)	12.18 (brs)
6	CH	-	-	6.84 (s)
7	OCH_3	3.71 (s)	3.69 (s)	3.92(s)
8	CH	6.95 (s)	6.93 (s)	7.04 (s)

3.4 Plant Growth Regulator Activity of Isolated Compounds

In order to reach the goal of this research, all isolated substances were subjected to plant growth regulator test. The results of this activity against *B. pekinensis* Rupr. were presented in Table 3.6 and Figures 3.1-3.2.

Table 3.6 Inhibitory effect of isolated substances from the culture of *A. porri* on growth of *B. pekinensis* Rupr.

Substance	**Part of** *B. pekinensis*	**% Inhibition at concentration (ppm)**		
		10	**100**	**1,000**
Compound **1**	Shoot	12.98 ± 8.71	30.16 ± 5.54	100 ± 0.00
	Root	25.08 ± 1.99	86.50 ± 6.27	100 ± 0.00
Compound **2**	Shoot	1.85 ± 5.42	22.80 ± 11.15	67.04 ± 14.13
	Root	13.30 ± 3.00	16.51 ± 3.31	85.66 ± 1.71
Compound **3**	Shoot	10.94 ± 7.25	20.18 ± 8.99	83.01 ± 14.01
	Root	35.62 ± 17.34	57.25 ± 9.89	97.40 ± 2.63
Compound **4**	Shoot	7.49 ± 10.06	9.98 ± 8.23	10.10 ± 11.67
	Root	-1.76 ± 4.34	16.06 ± 2.80	25.92 ± 4.14
Compound **5**	Shoot	9.29 ± 8.00	16.36 ± 8.07	19.53 ± 7.40
	Root	-8.78 ± 6.41	20.82 ± 9.61	9.28 ± 11.58
Round up®	Shoot	14.65 ± 6.53	28.44 ± 7.03	73.44 ± 13.70
	Root	72.55 ± 2.96	83.94 ± 2.06	93.13 ± 2.58

The effect of isolated substances were classified as inhibition (+) and promotion (-).

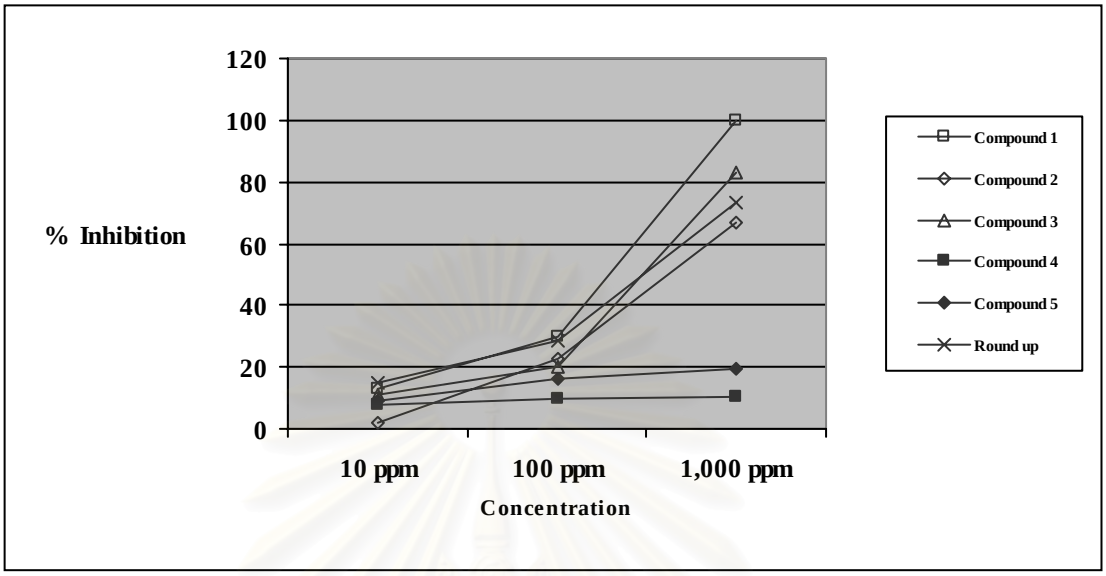


Figure 3.1 Inhibitory effect of isolated substances from the culture of *A. porri* on the shoot length of *B. pekinensis* Rupr.

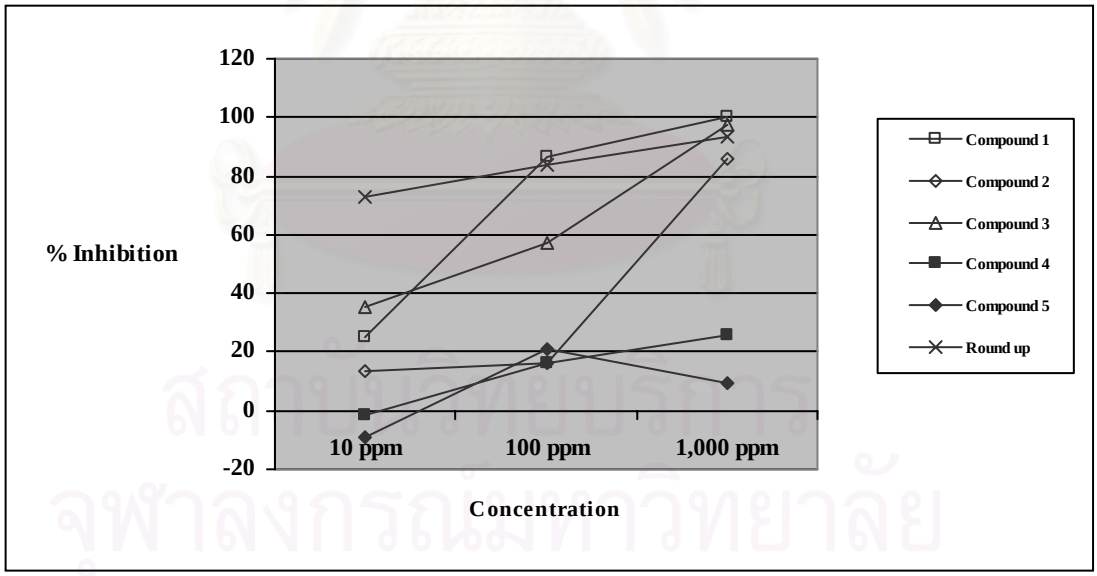


Figure 3.2 Inhibitory effect of isolated substances from the culture of *A. porri* on the root length of *B. pekinensis* Rupr.

The results of plant growth inhibition activity against *B. pekinensis* Rupr. seedling (lettuce) showed that altersolanol A was the strongest root growth inhibition at 1,000 and 100 ppm which was higher activity than round up®. Altersolanol B and C showed the inhibition activity close to altersolanol A. In addition, altersolanol B and C was higher inhibition against lettuce root than its shoot. The growth inhibition of altersolanol A, B and C were decreased by polarity. The low activity was compound **4** and **5** which did not show significant inhibitory effect between substances. At highest concentration (1,000 ppm), compound **4** exhibited stronger growth inhibition than **5**. On the other hand, compound **5** exhibited higher activity on lettuce seedling at 100 and 10 ppm. In addition, the shoot growth inhibition of herbicide clearly appeared at only highest concentration, but the root growth inhibition was less difference when the concentration was decreased.

In addition, all substances were further bioassay for plant growth inhibition against *Echinochloa crus-galli* (L.) Beauv. seedlings, as a monocotyledon, which is a common and very important weed of the most agricultural areas of the world. The results of inhibitory effect are showed in Table 3.7 and Figures 3.3-3.4.

Table 3.7 Inhibitory effect of isolated substances from the culture of *A. porri* on the growth of *E. crus-galli* (L.) Beauv.

Substance	**Part of** *E. crus-galli*	**% inhibition at concentration (ppm)**		
		10	**100**	**1,000**
Compound **1**	Shoot	3.87 ± 7.60	57.34 ± 11.89	100 ± 0.00
	Root	-26.42 ± 10.54	82.18 ± 3.6	100 ± 0.00
Compound **2**	Shoot	14.01 ± 8.65	38.70 ± 9.57	89.78 ± 7.98
	Root	-4.84 ± 10.69	61.36 ± 3.37	95.95 ± 5.20
Compound **3**	Shoot	16.16 ± 9.84	37.97 ± 16.97	73.98 ± 7.10
	Root	19.59 ± 7.56	72.78 ± 10.76	96.25 ± 5.70
Compound **4**	Shoot	15.17 ± 7.23	20.96 ± 5.24	17.75 ± 6.89
	Root	-10.16 ± 12.56	-3.52 ± 7.43	6.16 ± 5.06
Compound **5**	Shoot	16.26 ± 8.13	23.24 ± 6.51	17.81 ± 4.15
	Root	-12.42 ± 7.77	-2.02 ± 7.75	2.87 ± 6.46
Round up®	Shoot	74.67 ± 12.48	100.00 ± 0.00	100.00 ± 0.00
	Root	85.26 ± 9.75	100.00 ± 0.00	100.00 ± 0.00

The effect of isolated substances were classified as inhibition (+) and promotion (-).

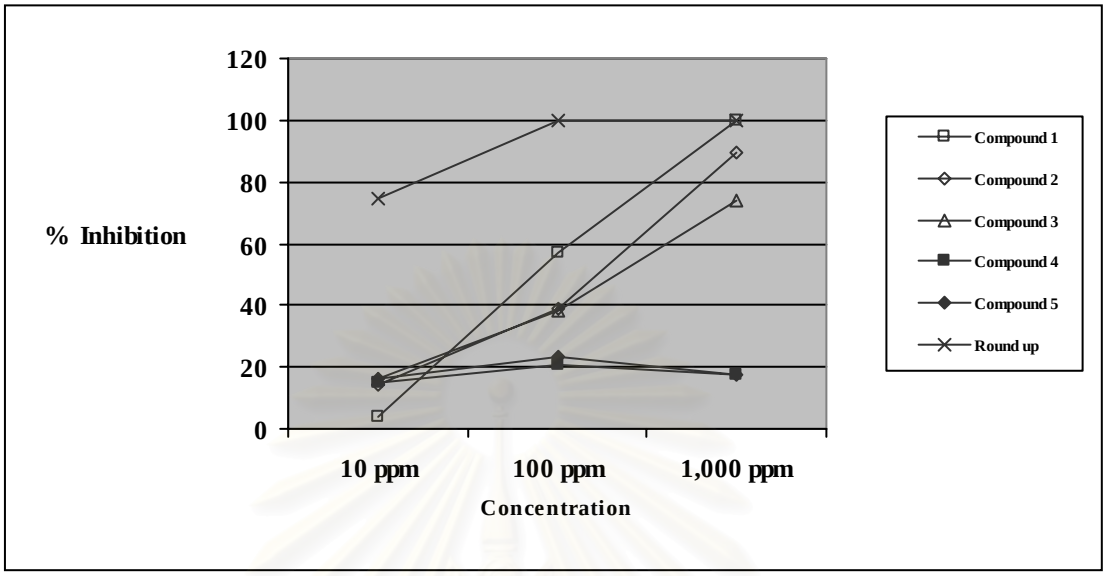


Figure 3.3 Inhibitory effect of isolated substances from the culture of *A. porri* on the shoot length of *E. crus-galli* (L.) Beauv.

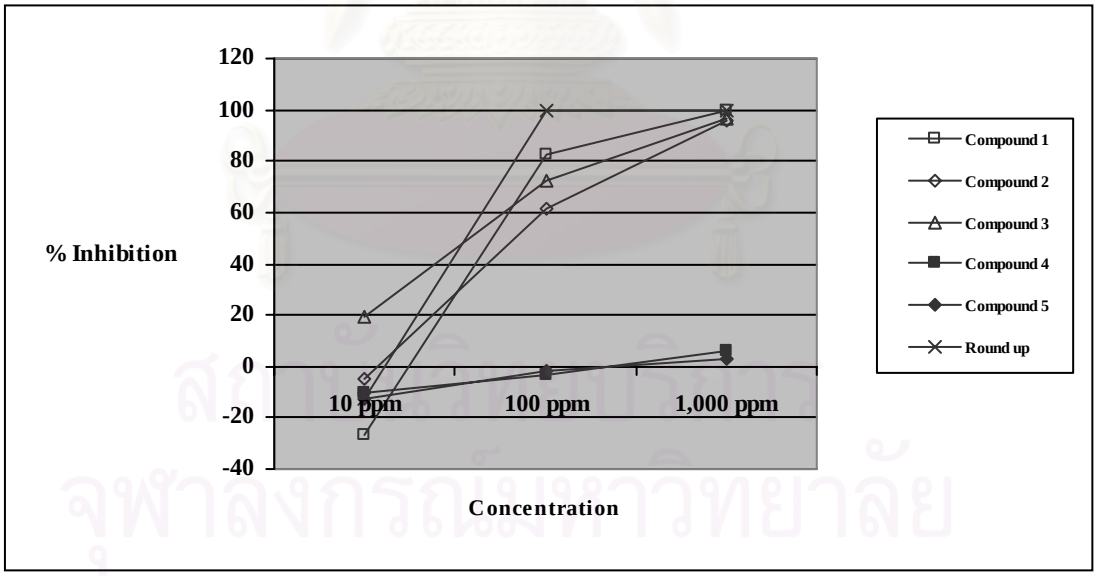


Figure 3.4 Inhibitory effect of isolated substances from the culture of *A. porri* on the root length of *E. crus-galli* (L.) Beauv.

From the results of plant growth inhibition activity against *E. crus-galli* (L.) Beauv. seedlings, altersolanol A still showed the strongest activity against growth of seedlings. Shoot and root growth of seedling were inhibited at high concentrations (1,000 and 100 ppm). The moderate growth inhibition was altersolanol C and B, respectively. Altersolanol A inhibited root growth better than shoot growth, the inhibition value was very similar with herbicidal inhibitory value at 1,000 ppm. In addition, the results showed weak inhibitory effect of growth seedling in compound **4** and **5**. The slightly shoot growth inhibitions were exhibited at all concentrations which were not significant effect between substances and concentrations. The effect of compound **4** and **5** on root growth showed weak inhibitory effect at 1,000 ppm. The slightly promotion of root growth was observed at 100 and 10 ppm.

From the results of activity against both *B. pekinensis* Rupr. and *E. crus-galli* (L.) Beauv. seedlings, showed the property of altersolanol series to regulated growth of plant. Altersolanol A exposed highly activity against growth seedling of monocotyledon and dicotyledon plants. The inhibition of root growth in monocotyledon plant was better. The activity of three compounds, altersolanol A, B and C, depended on polarity. On the other hand, the structure-like altersolanol A of compound **4** and **5** suggested that the inhibitory level was dropped by increasing of polarity from altersolanol A.

3.5 Discussion

The plant growth inhibitory level of altersolanol series, especially altersolanol A, B and C, may caused by the altersolanol series-like electron transport inhibitor of plant respiration (Haraguchi et al., 1996). The strongest respiratory inhibiting agent was altersolanol A, the moderate inhibitors were altersolanol C and B, respectively. These results suggested that the hydroxy group at C-5 of altersolanols may be essential to their role as electron acceptors and that the numbers of hydroxy groups at C-1, C-3, and C-5 may contribute to these activities. The results of plant growth regulators against both monocotyledon and dicotyledon, may be explained by the respiratory inhibitor relationship structure of altersolanol series. On the other hand, the difference of inhibitory effect between altersolanol A and compound **4** and **5**, showed different structure by increasing of hydroxy group and rearrangement of methoxy on aromatic ring (ring A) which affected to decrease plant growth regulation activity. Difference of structure between active compound, altersolanol A, B and C, and low active compound, compound **4** and **5** which exhibited position of important substitute group on ring A. The structural of active compound showed MeO-7 was important substituent which led the effect against growth seedling to high inhibitory activity. Inhibitory effect of active compound may caused be number of hydroxy groups on ring C which affected to altersolanol A was the strongest activity. The moderate activity were altersolanol C and B, respectively. The inhibitory level between altersolanol A and Round up® in monocotyledon and dicotyledon were not more different at high concentration. In addition, Roundup® was a non-selective herbicide (Daruich et al., 2001) which used in bioactivity test that contained isopropylamine salt and N-(phosphonomethyl) glycine. The N-(phosphonomethyl) glycine or glyphosate acted by preventing the synthesis of aromatic amino acid (Hopkins, 1999), which in turn blocked the synthesis of protein, auxin hormone and other important. The exhibition of inhibitory effect of herbicide was another pathway from altersolanol A, although the capacity of inhibition was not more different at high concentration.

3.6 Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C-NMR spectra were run on a Fourier Transform Nuclear Magnetic Resonance Spectrometer of Varian model Mercury+ 400. TLC were performed on precoated Merk silica gel 60 F254 (0.25 mm-thick layer). HPLC was performed on Water model Delta 600 with a Water Nova-Pak RP-C_{18} (8×100) column and Water model 2996 Photodiode Array detector. Chromatotron (model 7924 T, Harrison Research) on a silica gel plate of 1 mm thickness was performed on centrifugal thin layer chromatography. Adsorbents used for isolation were silica gel 60 Merck, cat. No.7731, 7734 and 7749 were used for quick column chromatography, column chromatography and chromatotron, respectively.

Altersolanol A (**1**): deep red solid

^{1}H NMR (400 Hz, DMSO-d_6), 12.18 (1H, brs, OH-5), 7.04 (1H, s, H-8), 6.84 (1H, s, H-6), 5.72 (1H, d, *J* = 6 Hz, OH-4), 5.07 (1H, d, *J* = 5.2 Hz, OH-1), 4.93 (1H, d, *J* = 6.4 Hz, OH-3), 4.50 (2H, overlap), 4.34 (1H, d, *J* = 4.8 Hz, H-1), 3.92 (3H, s, OMe-7), 3.66 (1H, t, *J* = 6.4, 6.8 Hz, H-3), 1.26 (3H, s, Me-2), ^{13}C NMR (400 Hz, DMSO-d_6), see Table 3.2.

Altersolanol B (**2**): deep red powder

^{1}H NMR (400 Hz, DMSO-d_6), 12.21 (1H, brs, OH-5), 7.00 (1H, d, *J* = 2.7 Hz, H-8), 6.76 (1H, d, *J* = 2.7 Hz, H-6), 4.77 (1H, d, *J* = 5.5 Hz, OH-3), 4.44 (1H, s, OH-2), 3.85 (3H, s , OMe-7), 3.52 (1H, m, H-3), 2.51-2.67 (4H, m), 1.12 (3H, s, Me-2).

Altersolanol C (**3**): deep red solid

^{1}H NMR (400 Hz, DMSO-d_6), 12.15 (1H, brs, OH-5), 7.00 (1H, d, *J* = 2.1 Hz, H-8), 6.78 (1H, d, *J* = 2.1 Hz, H-6), 5.50 (1H, d, *J* = 6.9 Hz, OH-1), 4.67 (1H, d, *J* = 6.9 Hz, H-1), 4.38 (1H, s, OH-2), 4.30 (1H, d, *J* = 6.2 Hz, OH-3), 3.87 (3H, s, OMe-7), 3.70 (1H, m, H-3), 2.76 (1H, dd, *J* = 18.1, 6.5 Hz, OH-4), 2.31 (1H, dd, *J* = 19.2, 9.6 Hz, H-4), 1.23 (3H, s, Me-2).

Compound 4: deep red solid

^{1}H NMR (400 Hz, DMSO-d_6), 13.11 (1H, brs, OH-5), 6.95 (1H, s, H-8), 5.69 (1H, d, *J* = 5.1 Hz, OH-4), 5.12 (1H, d, *J* = 3.9 Hz, OH-1), 4.91 (1H, d, *J* = 5.1 Hz, OH-3), 4.48 (2H, overlap), 4.08 (1H, d, *J* = 5.1 Hz, H-1), 3.71 (3H, s, OMe-7), 3.57 (1H, t, *J* = 5.1, 5.4 Hz, H-3), 1.16 (3H, s, Me-2).

Compound 5: deep red solid

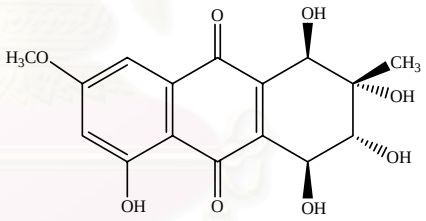
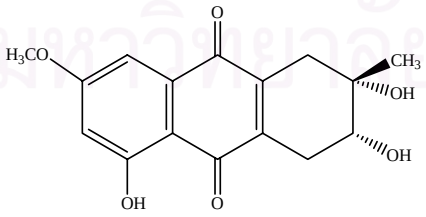
^{1}H NMR (400 Hz, DMSO-d_6), 13.11 (1H, brs, OH-5), 6.93 (1H, s, H-8), 5.69 (1H, d, *J* = 6.8 Hz, OH-4), 5.08 (1H, d, *J* = 3.9 Hz, OH-1), 4.89 (1H, d, *J* = 6.4 Hz, OH-3), 4.48 (2H, overlap), 4.13 (1H, d, *J* = 5.1 Hz, H-1), 3.67 (3H, s, OMe-7), 3.60 (1H, t, *J* = 6.4, 7.2 Hz, H-3), 1.15 (3H, s, Me-2).

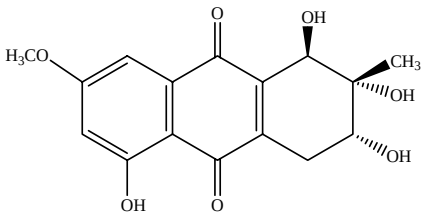
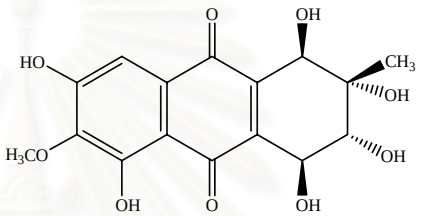
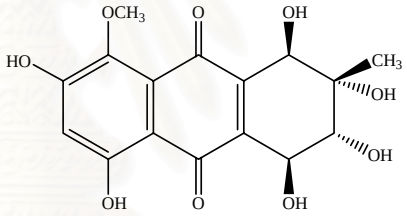
CHAPTER IV

CONCLUSION

The preliminary results for plant growth regulators activity revealed that the liquid culture extract of *Alternaria porri* displayed significant activity. This fungus was selected for chemical constituent studies. Bioassay-guided isolation exhibited that ethyl acetate extract was the most active against seedling growth of *B. pekinensis* Rupr. at 1,000 ppm

Five compounds were isolated and purified from the liquid culture extract of *A. porri*. The chemical structures were characterized by spectroscopic data. All isolated compounds are summarized as shown.

Compound **1**	 altersolanol A
Compound **2**	 altersolanol B

Compound **3**	 altersolanol C
Compounds **4** and **5**	 

In plant growth regulators bioassay against *B. pekinensis* Rupr. seedling, altersolanol A showed highest inhibitory effect, which completely inhibited both root and shoot growth seedling at concentration of 1,000 ppm. Altersolanols C and B inhibited root growth of lettuce seedling at concentration of 1,000 ppm by 97.15 and 85.53 %, respectively. In addition, the inhibitory effect against root and shoot growth of herbicide and altersolanol A were slightly different at 100 ppm. Altersolanols C and B showed moderate inhibition on root and shoot growth.

Furthermore, inhibitory effects against *E. crus-galli* (L.) Beauv. seedling of the isolated compounds were comparable to those against *B. pekinensis* Rupr, in which altersolanol A was the most active one.

Unlike compound **1-3**, **4** and **5** showed weak inhibition on both monocotyledon and dicotyledon. In fact, they slightly enhanced plant growth, particularly monocotyledon plant.

Interestingly, the inhibitory level between altersolanol A and herbicide in dicotyledon were not different which predicted to use altersolanol A for dicotyledon herbicide to inhibited growth.

Proposal for Future Work

From the result of plant growth regulators, altersolanol-derived substances showed significant inhibitory effect against both monocotyledon and dicotyledon plants. The interesting future work related to this research would be further examination on other monocotyledon and dicotyledon plants problematic to in Thai agriculture. Moreover, the investigation of plant growth regulator activity has been well known as a preliminary indicator that could be used for further study on other methods such as pot test and field test. An SAR study of natural molecules such as altersolanol A may be interesting to develop as herbicides.

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APPENDICES

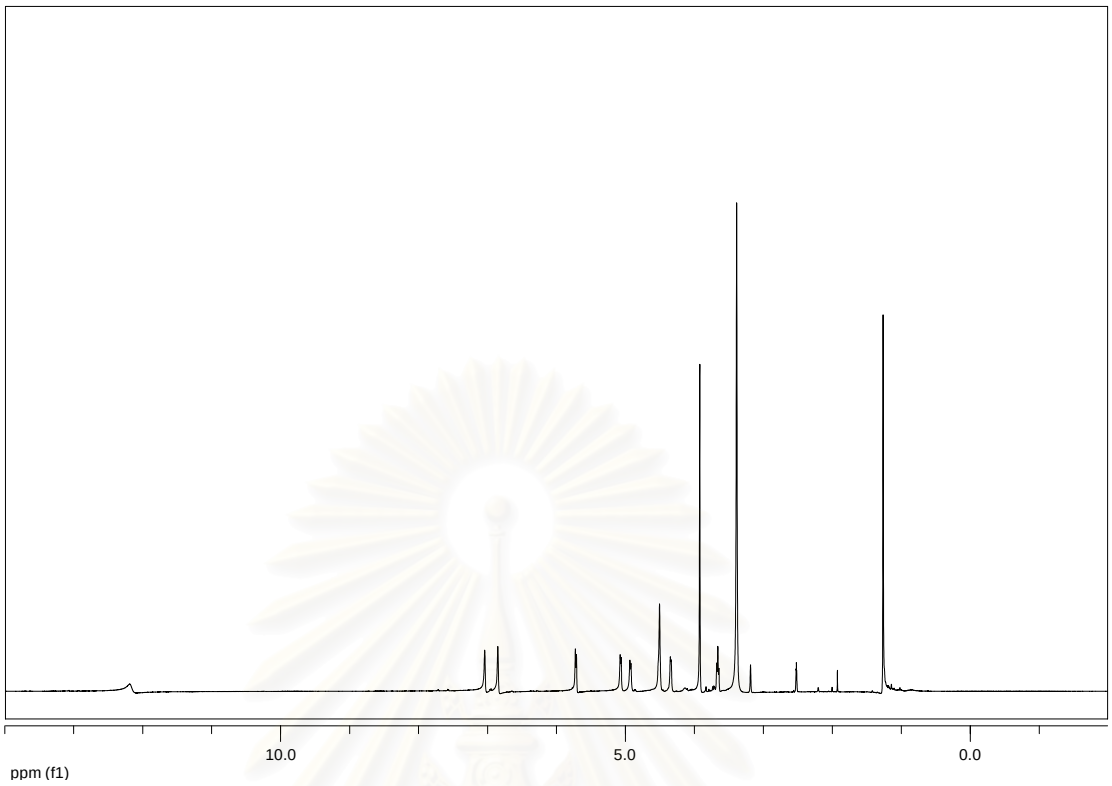

Figure 1 The ^{1}H NMR spectrum of compound **1** in DMSO-d_6

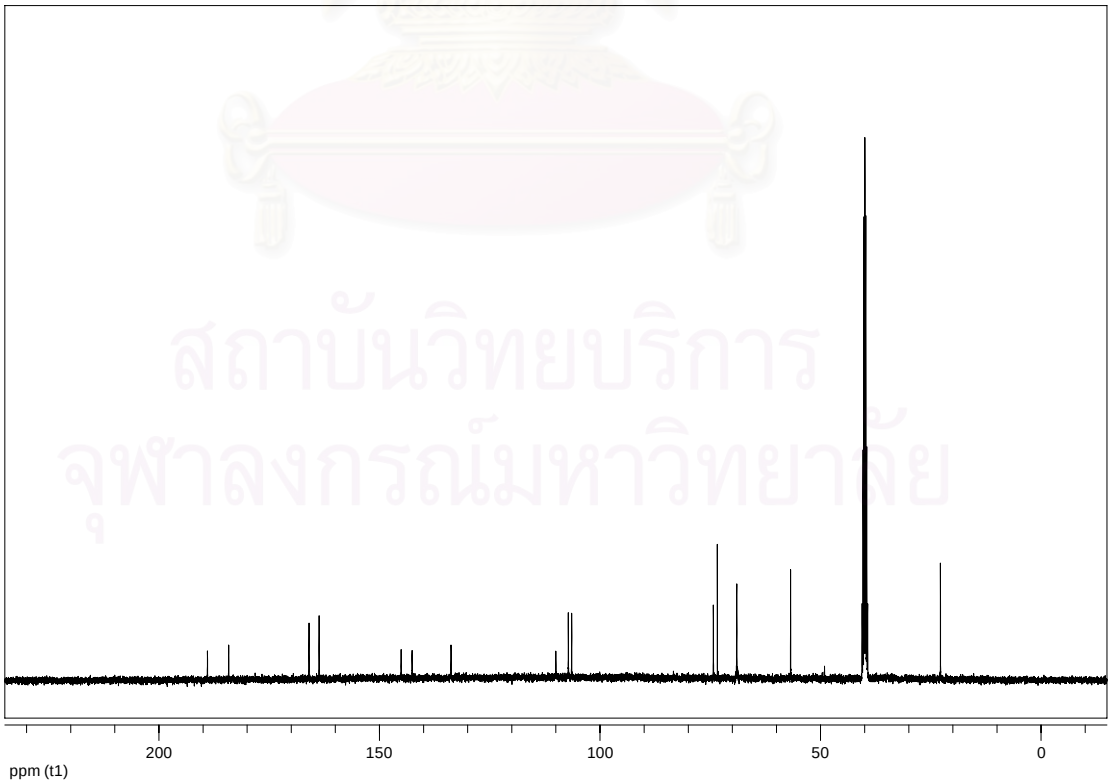

Figure 2 The ^{13}C NMR spectrum of compound **1** in DMSO-d_6

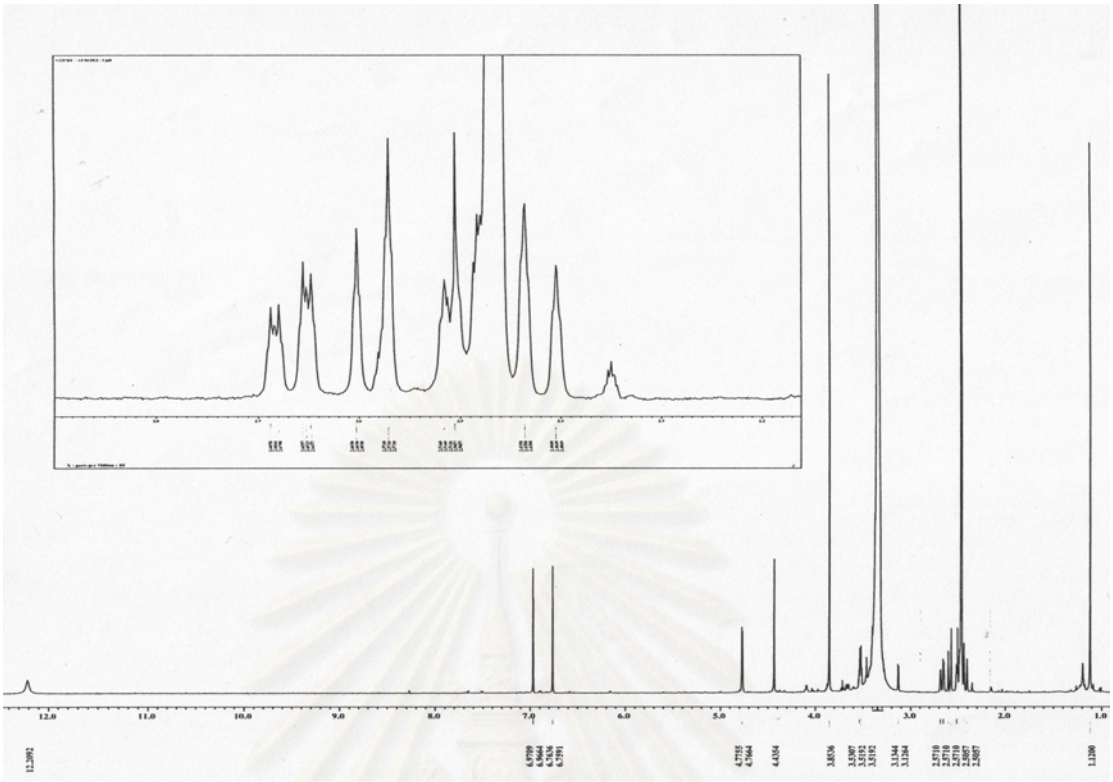

Figure 3 The ^{1}H NMR spectrum of compound **2** in DMSO-d_6

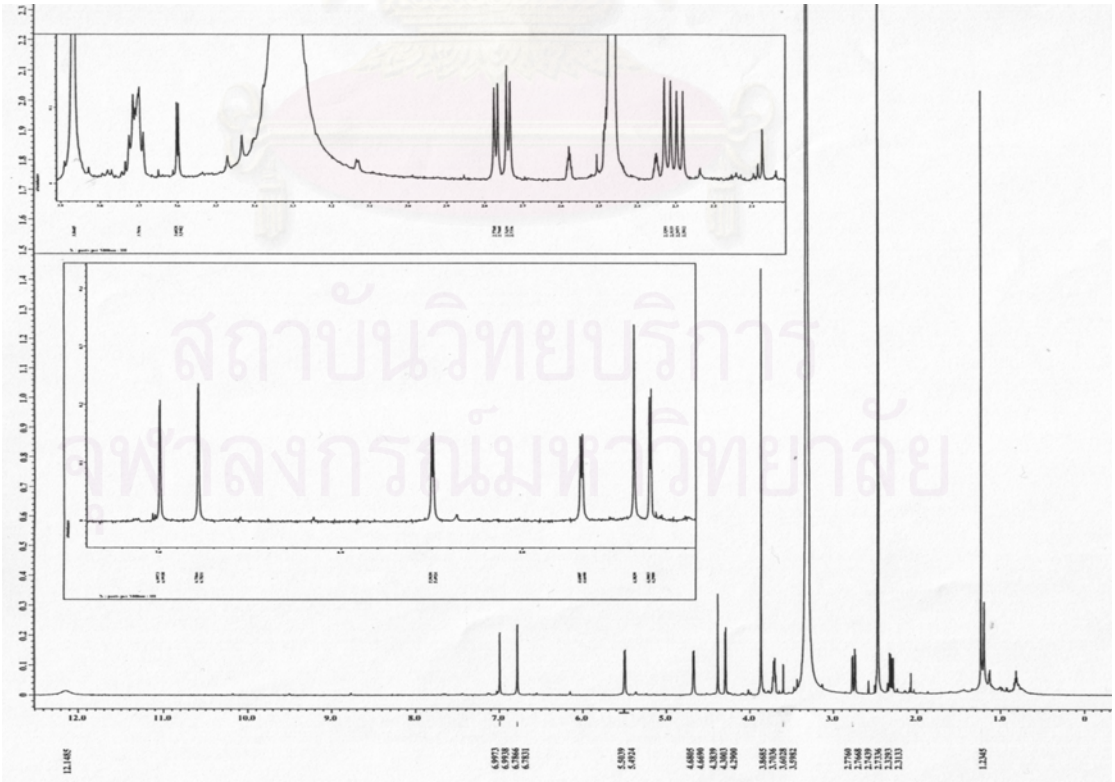

Figure 4 The ^{1}H NMR spectrum of compound **3** in DMSO-d_6

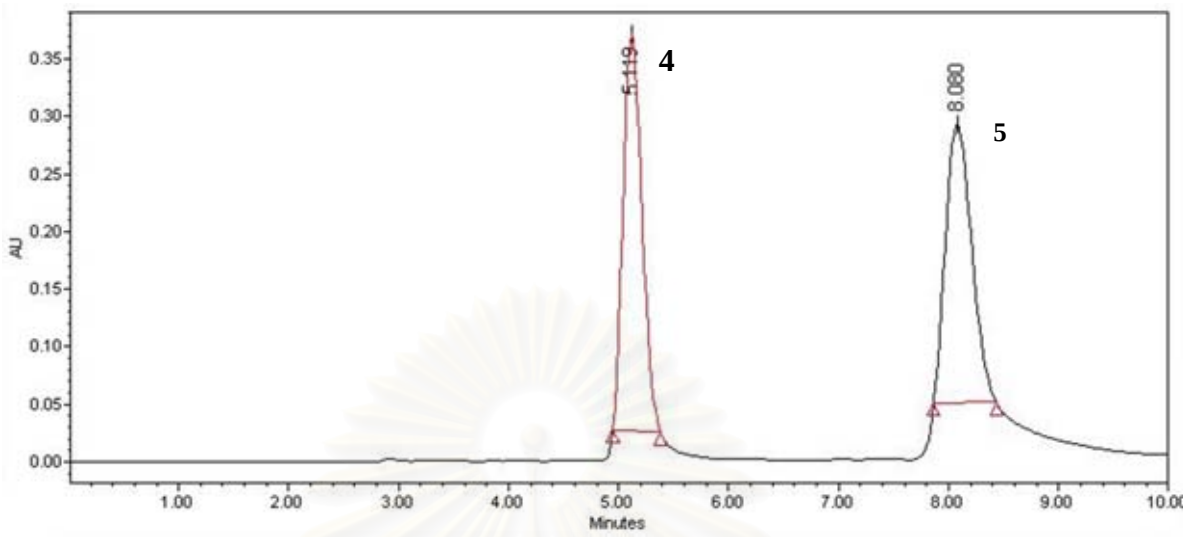

Figure 5 HPLC chromatogram of compound **4** and **5**

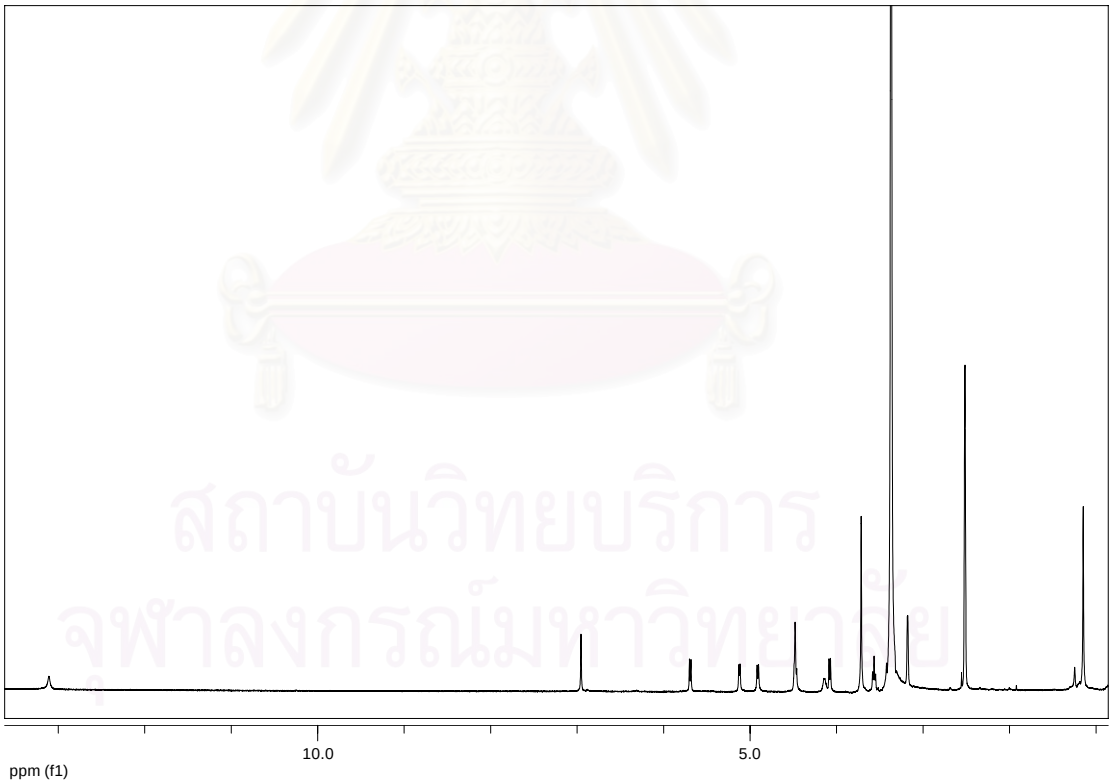

Figure 6 The ^{1}H NMR spectrum of compound **4** in DMSO-d_6

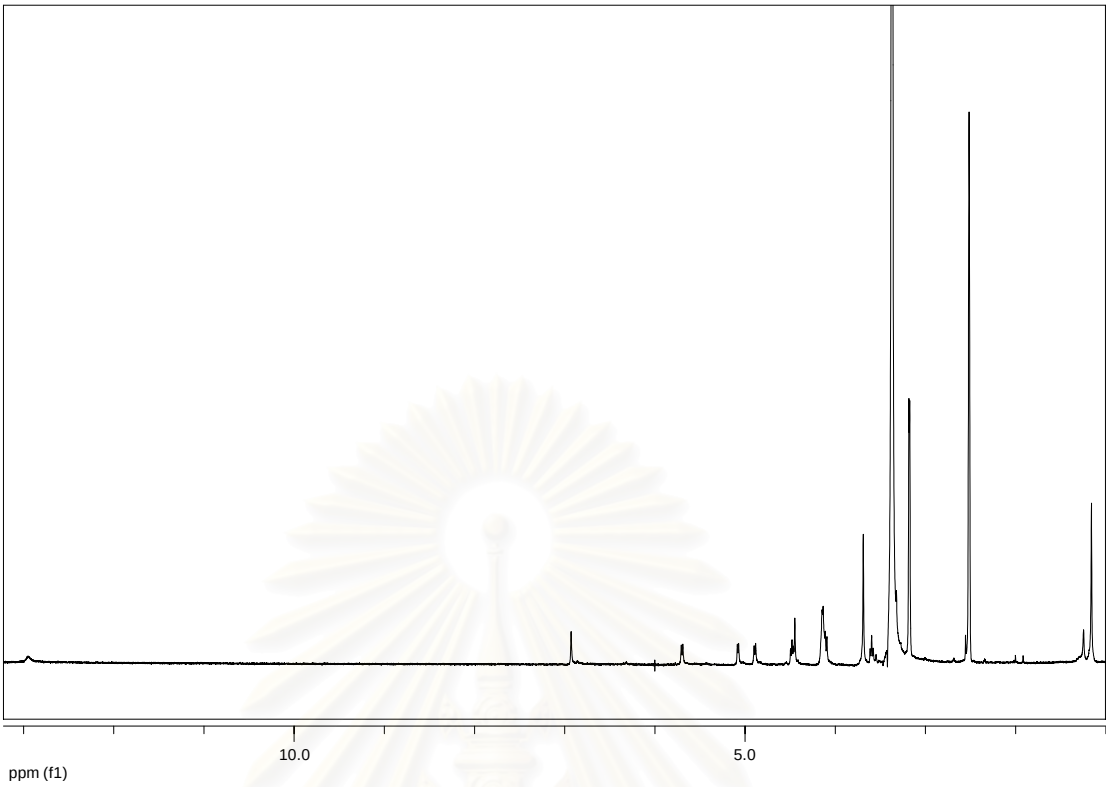

Figure 7 The ^{1}H NMR spectrum of compound **5** in DMSO-d_6

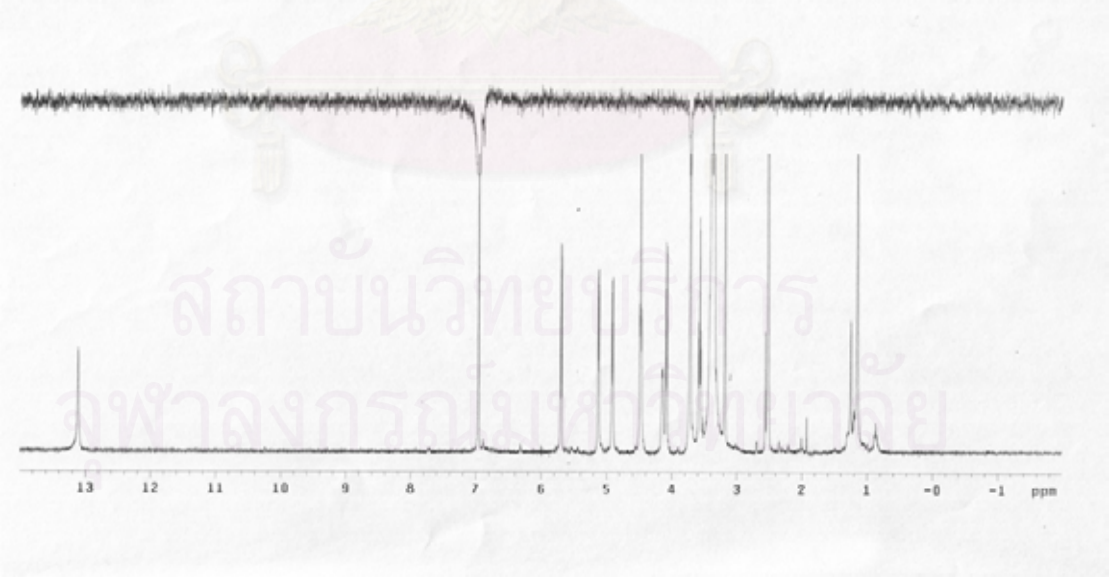

Figure 8 The spectrum of irradiation at aromatic proton of compound **4** in DMSO-d_6

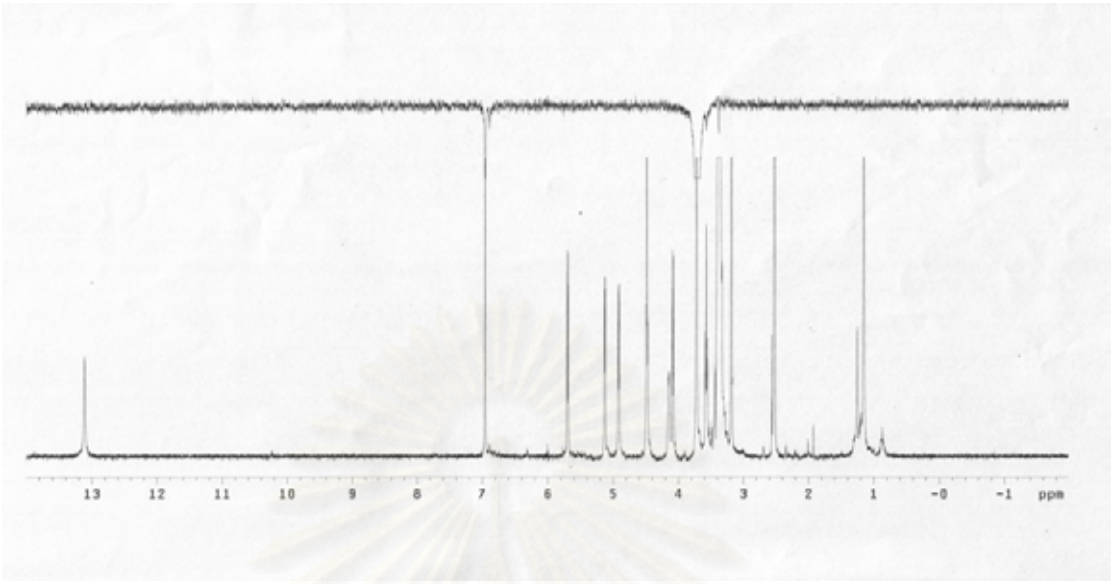

Figure 9 The spectrum of irradiation at methoxy group of compound **4** in DMSO-d_6

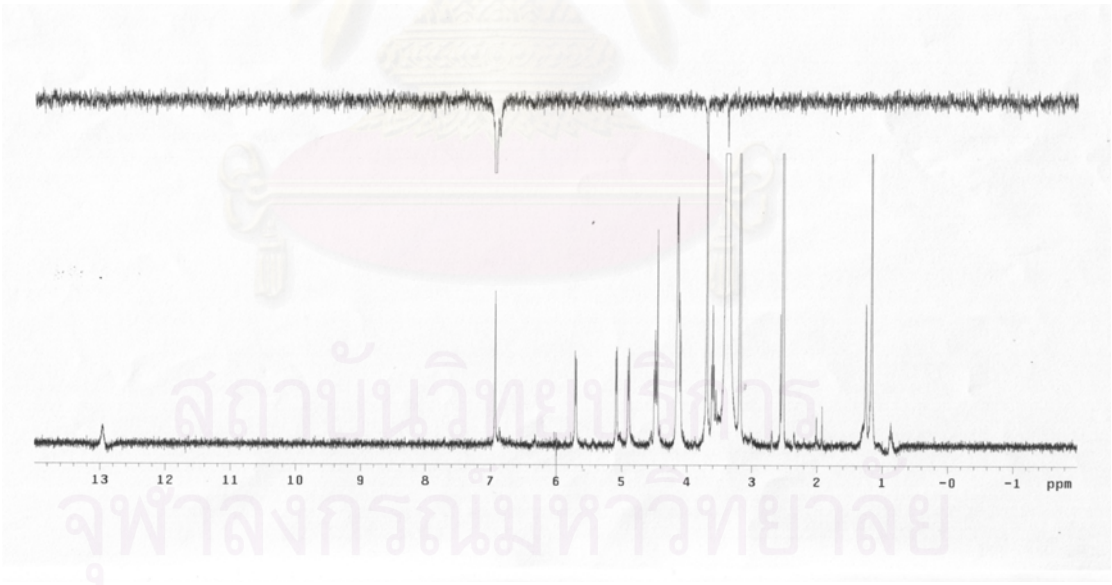

Figure 10 The spectrum of irradiation at aromatic proton of compound **5** in DMSO-d_6

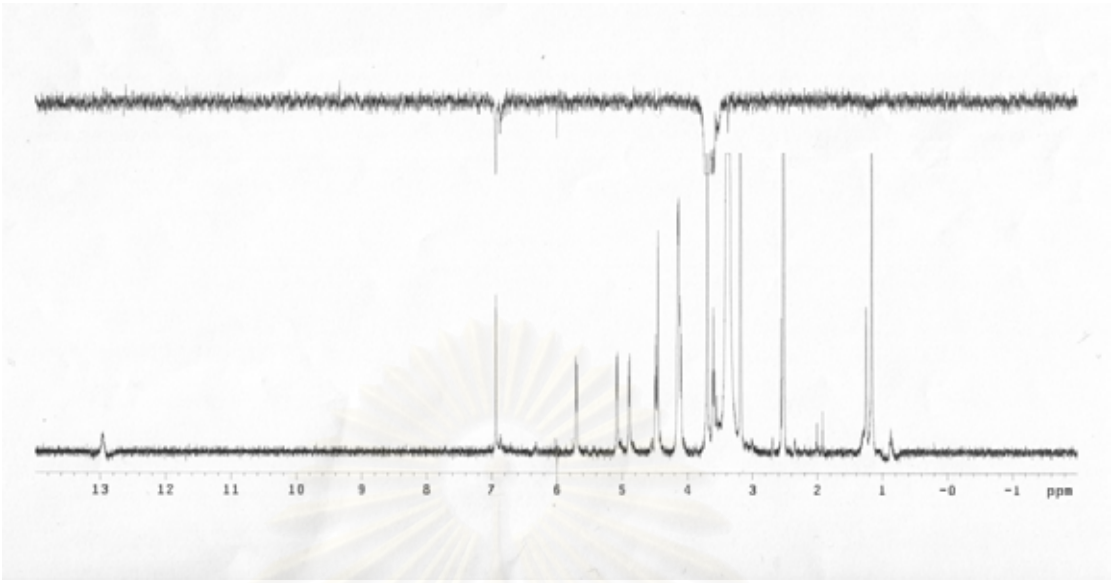

Figure 11 The spectrum of irradiation at methoxy group of compound **5** in DMSO-d_6

Table A Homogeneous groups of inhibitory effect of isolated substances on root length of *B. pekinensis* Rupr. at 1,000 ppm analyze by Duncan' One-way Anova test

Concentration	Homogeneous groups (alpha = 0.05)				
	1	2	3	4	5
Compound **4**	9.2773				
Compound **5**		25.9174			
Compound **2**			85.6584		
Round up®				93.1259	
Compound **3**					97.3966
Compound **1**					100.0000
Sig.	1.000	1.000	1.000	1.000	0.181

Table B Homogeneous groups of inhibitory effect of isolated substances on root length of *E. crus-galli* (L.) Beauv. at 1,000 ppm analyze by Duncan' One-way Anova test

Concentration	Homogeneous groups (alpha = 0.05)		
	1	2	3
Compound **4**	2.8748		
Compound **5**	6.1655		
Compound **2**		95.9520	
Compound **3**		96.2518	96.2518
Compound **1**			100.0000
Round up®			100.0000
Sig.	0.080	0.872	0.059

VITA

Sakesan Lorsakunrat was born on August 23, 1979 in Surin province, Thailand. He obtained a Bachelor Degree of Science, majoring in Genetics from Chulalongkorn University, Bangkok, Thailand in 2001. Since 2002, he has been a graduate student studying in Biotechnology program, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. During the course of study, he was supported by a research grant for his Master degree's thesis from Graduate School, Chulalongkorn University.