องค์ประกอบทางเคมีของส่วนสกัดใบและรากจากต้นหนามพุงดอ *Azima sarmentosa* Benth
และฤทธิ์ทางชีวภาพ

นายพันธ์ญา สุนินทบูรณ์

18580154

CHEMICAL CONSTITUTENTS OF LEAF AND ROOT EXTRACTS FROM

Azima sarmentosa Benth AND THEIR BIOLOGICAL ACTIVITIES
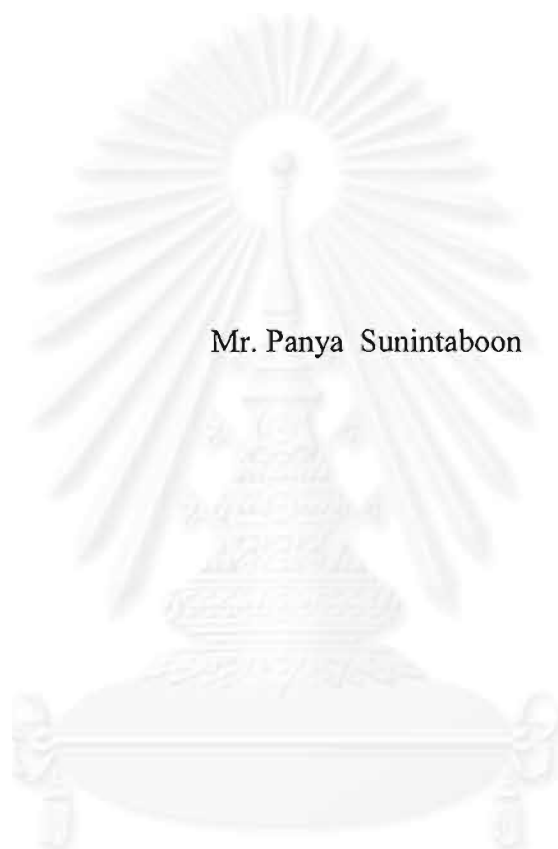
Mr. Panya Sunintaboon

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Chemistry

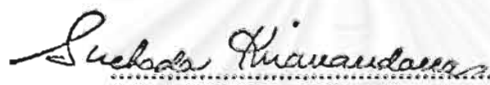Department of Chemistry

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Thesis Title   Chemical Constituents of Leaf and Root Extracts from

                       Azima sarmentosa Benth and Their Biological Activities

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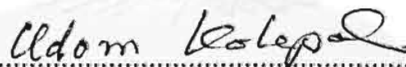Thesis Advisor   Associate Professor Udom Kokpol, Ph.D.

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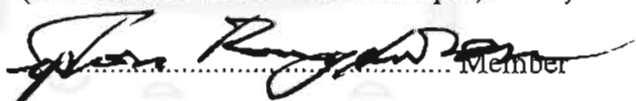
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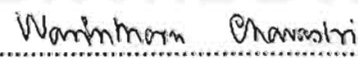
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พันธ์ญา สุนินทบูรณ์ : องค์ประกอบทางเคมีของส่วนสกัดใบและราก จากต้นหนามพุงดอ *Azima sarmentosa* Benth และฤทธิ์ทางชีวภาพ (CHEMICAL CONSTITUENTS OF LEAF AND ROOT EXTRACTS FROM *Azima sarmentosa* Benth AND THEIR BIOLOGICAL ACTIVITIES) อ. ที่ปรึกษา : รองศาสตราจารย์ ดร. อุดม ก๊กผล; 106 หน้า. ISBN 974-332-757-6.

ในการศึกษาองค์ประกอบทางเคมี ของส่วนสกัดใบ และรากจากต้นหนามพุงดอ (*Azima sarmentosa* Benth) สามารถแยกสารบริสุทธิ์ ได้ 3 ชนิด จากส่วนสกัดใบ คือ taraxerone, tarxerol และ ไตรเทอร์พีนอยด์ I และ สารบริสุทธ์ 7 ชนิด จากส่วนสกัดราก คือ ไตรเทอร์พีนอยด์ II, stigmasterol, 1H-indole-3-carboxaldehyde, 1-methoxy-indole-3-carboxaldehyde, 1-methoxy-indole-3-acetonitrile, 5-hydroxymethyl furfuraldehyde และ stigmasteryl-3-O-β-D-glucopyranoside ซึ่งเป็นรายงานครั้งแรกเกี่ยวกับองค์ประกอบทางเคมีที่พบในพืชนี้ 1-methoxy-indole-3-carboxaldehyde และ 1-methoxy-indole-3-acetonitrile แสดงความเป็นพิษต่อไบรน์ชริมพ์ (*Artemia salina* Linn.) ด้วยค่า LC_{50} 0.09 และ 9.24 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ นอกจากนี้ สารทั้งสองชนิดนี้ รวมทั้ง 1H-indole-3-carboxaldehyde และ ไตรเทอร์พีนอยด์ II มีฤทธิ์เป็น antioxidant ด้วย

ภาควิชา	เคมี	ลายมือชื่อนิสิต	
สาขาวิชา	เคมี	ลายมือชื่ออาจารย์ที่ปรึกษา	
ปีการศึกษา	2542	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม	

In the investigation of chemical constituents of the leaf and the root extracts from *Azima sarmentosa* Benth, three compounds were isolated from the leaf extract: taraxerone, taraxerol and unidentified triterpenoid I. Seven compounds were isolated from the root extract: unidentified triterpenoid II, stigmasterol, 1H-indole-3-carboxaldehyde, 1-methoxy-indole-3-carboxaldehyde, 1-methoxy- indole-3-acetonitrile, 5-hydroxymethyl furfuraldehyde and stigmasteryl-3-O-β-D-glucopyranoside. This is the first report of the chemical constituents of this plant. 1-methoxy- indole-3-carboxaldehyde and 1-methoxy- indole-3-acetonitrile exhibited cytotoxic lethality on brine shrimp (*Artemia salina* Linn.) with LC$_{50}$ 0.09 and 9.24 respectively. Both compounds, 1H-indole-3-carboxaldehyde and unidentified triterpenoid II also showed antioxidant activity.

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

สาขาวิชา............เคมี............ ลายมือชื่ออาจารย์ที่ปรึกษา..

ปีการศึกษา............2542............ ลายมือชื่ออาจารย์ที่ปรึกษาร่วม..

ACKNOWLEDGEMENT

Contents

List of Figures

List of Tables

List of Schemes

List of Abbreviations

br	broad	mL	milliliter (s)
°C	degree Celsius	m.p.	melting point
cm^{-1}	unit of wavenumber	MW	molecular weight
dd	double of doublet	NMR	nuclear magnetic resonance
d	doublet (NMR)	ppm	part per million
td	triple of doublet (NMR)	q	quartet (NMR)
mg	milligram (s)	s	strong (IR)
Hz	hertz	s	singlet (NMR)
IR	infrared	t	triplet (NMR)
J	coupling constant	w	weak (IR)
LC_{50}	concentration that caused 50% lethality	δ	chemical shift
w/w	weight by weight	%	percent
m	multiplet (NMR)	μg	microgram (s)
m	medium (IR)	μL	microliter (s)
Fig.	figure	Hex	hexane
CH_2Cl_2	dichloromethane	$CHCl_3$	chloroform
EtOAc	ethyl acetate	MeOH	methanol
R_f	retardation factor		

Chapter I
Introduction

It has been long thought that human can use plants in many ways and, because of biological diversity, human can obtain numerously valuable benefits from these natural resources. Fortunately, Thailand is located in the tropical region of the world and has abundant kinds of plants, especially herbs which are used as medicinal plants. Indigenous people have made use of plants in many ways: as food, and as medicine to treat diseases. In the past, however, they might have obtained benefits from plants accidentally and learnt about them without systematic methodology. The utilization of plants is now limited to a small group of people, especially in the far up country, and many people do not have the knowledge or courage to use them. The study of plants has several approaches: domestic thai medicine, pharmaceutical aspects, natural product chemistry, agrochemistry and phytochemistry, leading to increased benefits from plants. Many researchers have searched for medicinal plants to cure diseases which, nowadays, are increasing considerably.

Resulting from the systematic investigation, it has been revealed, for instance, that *Curcuma longa* Linn., tumeric which is a herbal plant in Thailand possesses curcumin which stimulates the release of musin to prevent ulcers.[1] Also, cassumunin[2] gained from this plant is antimalarial. *Catharanthus roseus* G.Don, has yielded the alkaloids, vinblastine which can treat leukemia, and vincristine which can treat Hodgkin's disease.[1] Furthermore, ethanolic extract of *Coccinia grandis* Linn, Voigt has played an important role in reducing sugar level in blood leading to minimizing diabetes.[1] The popularity of utilizing plants might be due to their less side-effects, reasonable cost and greater availability. In addition, consuming local medicinal plants can reduce the import of synthetic drugs from foreign countries. In agricultural benefit, it is possible to make use of useless weeds as plant-growth inhibitor or plant growth regulator. There is a report that gallic acid in *Phyllanthus niruri* Linn.[3], tropical weed in Thailand, can dramatically inhibit growth of rice which

is selected as a model of typical weed. The importance of the plants not only is as food or as medicine but, in the present, in agriculture and cosmetics industry as well.

Natural product chemistry is one of the approaches to searching for chemical constituents from natural plants and involves seeking their activities by bioassay tests and therapeutic applications. The criteria traditionally used to select plant to study could be the history of that plant in treating diseases, preliminary screening bioactivity tests, or literature surveys about chemical constituents and biological activities. Natural product researchers have the advantage in providing the basic information about structure and bioactivities of the substances which may have the potential as drugs (applied as the commercial drugs in the future or even used as crude drugs) or herbicides.

In terms of natural product chemistry research, this thesis is focused on searching for bioactive compounds from the tropical Thai plant. *Azima sarmentosa*, one of the indigenous Thai plants, was selected to study systematically.

A. sarmentosa, belonging to family Salvadoraceae, is broadly distributed in the tropical regions all over the world.[4] In Thailand, this plant is distributed in many areas such as central, north eastern and northern regions of the country. *A. sarmentosa* is rambling shrub, with axilliary spines. The leaves are opposite and it has characteristic as follows: entire flowers are small, dioecious, axillary, sestile, or on little-branched panicle in cluters or umbels; bracts are 0 or leaflike; bracteoles are linear and small. Calyx campanulate is 4-fid or irregularly 2-4-lobed. Petals are hypogynous.[5]

There have been several common names called in Thailand: *Phungdo* or *Naam phungdo* (Central), *Pittoh* (Chiang Mai) or *Khee-haet* (Shan-Northern).[6]

1.1 *Azima sarmentosa* as herbal medicines

A. sarmentosa has been used as the medicinal plant in Thailand. The roots are usually used and have a sour taste. It is utilized as an antipyretic and anti-inflammatory agent. Moreover, it can lessen the pain of wounds because of its cooling property. Also, it has been used to treat mumps.[6]
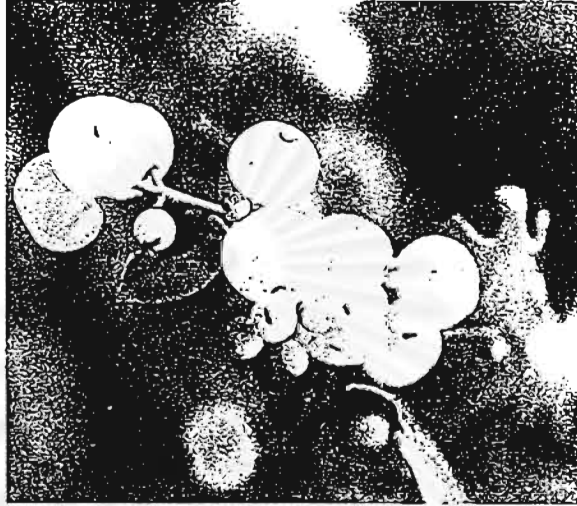
Fig. 1.1 Fruits and leaves of *A. sarmentosa* [7]

1.2 Chemical constituents found in Azima-genus plants

No detailed information about *A. sarmentosa* has been reported, but some reports have been made of *A. tetracantha.*

Rall et.al found dimeric piperidine alkaloids in the leaf extract of *A.tetracantha.* In that study, the new alkaloid, azimine (n=m=5), together with a minor non-crystalline alkaloid, azcarpine (n=5, m=7) and minute amounts of the closely related known carpaine (n=m=7) were isolated the leaves of *A.tetracantha.* Structures of these alkaloids are shown below: [8]

azimine (n=m=5)
azcarpine(n=5, m=7)
carpaine(n=m=7)
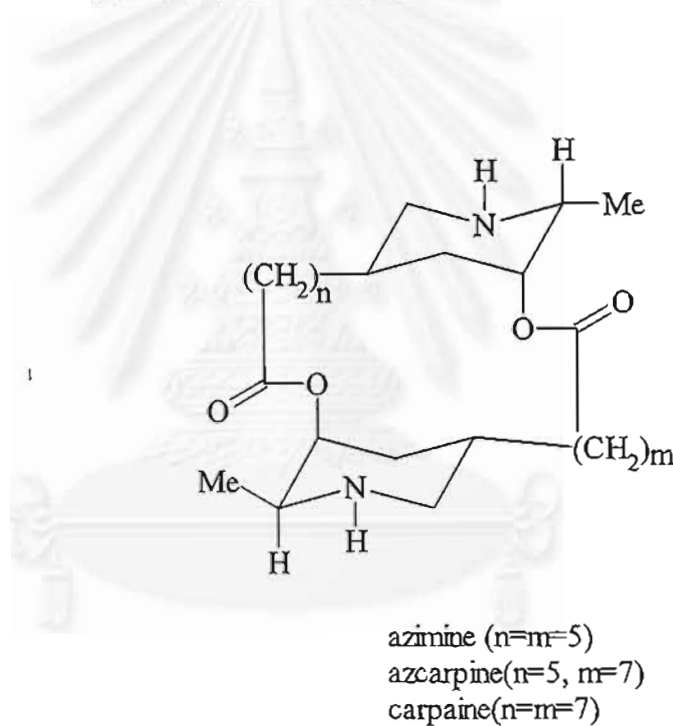
Fig. 1.2 Dimeric piperidine alkaloids

The occurrence of triterpenoids in *A. tetracantha* was also reported. With the traditional chromatography and spectroscopic identification, friedelin, glutinol, lupeol, and β-sitosterol were found. The structures are shown below: [9]

Friedelin

Glutinol

β-sitosterol

Lupeol
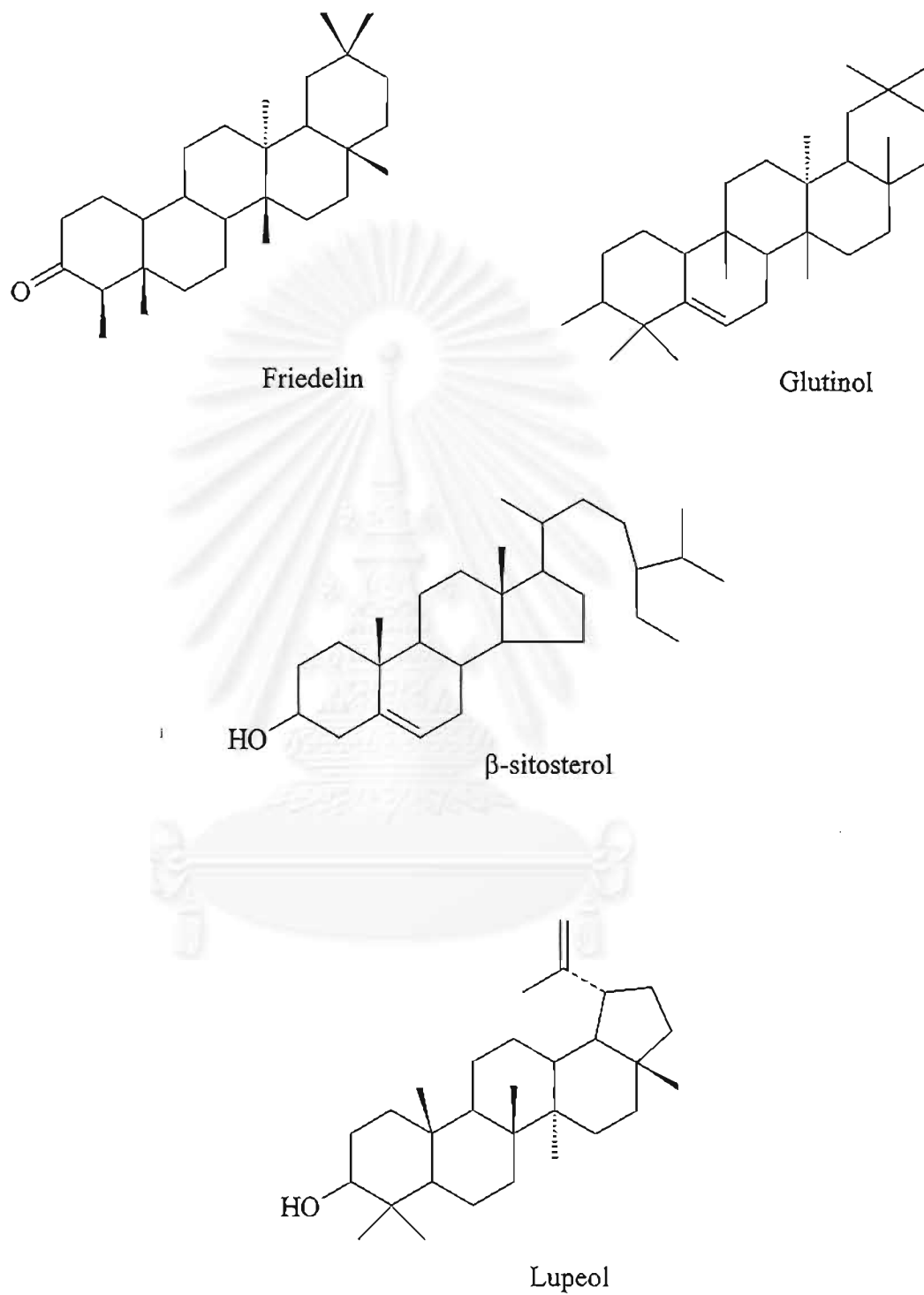
Fig. 1.3 Steroidal and triterpenoidal compounds found in

A. tetracantha

In addition, the acid components of *A. tetracantha* seed oil have been investigated. The seed oil of *A. tetracantha* contained the following fatty acids: myristic 0.2, palmitic 5.0, stearic 14.8, arachidic 6.7, behenic 2.4, oleic 31.8, linoleic 18.0, and eicosenic acid 21.1% by wt. [10]

$$CH_3(CH_2)_nCOOH$$
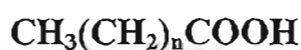
n=12 myristic acid

n=14 palmitic acid

n=16 stearic acid

n=18 arachidic acid

n=20 behenic acid (docosanoic acid)

$$CH_3(CH_2)_7CH=CH(CH_2)_nCOOH$$
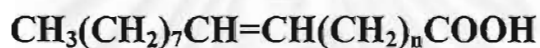
n= 7 oleic acid

n = 9 eicosenic acid

$$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_nCOOH$$

linoleic acid

A. tetracantha also contained ricinoleic acid (9.8%) and a novel cyclopropenoid fatty acid (9.6%), novel fatty acid, along with normal fatty acids. [11]

A flavonoid glycoside, isorhamnetin-3-O-rutinoside was isolated from the leaves of *A. tertracantha*. The isolation of this relatively rare glycoside is significant, because it has only been previously identified in 3 other plant sources, viz. *Narcissus tazetta*, *Lilium auratum*, and *Bupleurum ptundifolium*. [12]
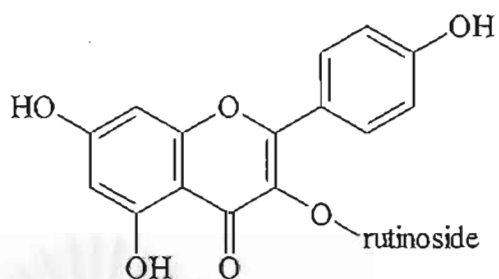
Isorhamnetin-3-O-rutinoside

Fig. 1.4 The structure of isorhamnetin-3-O-rutinoside

There was the only one report about the chemical constituents of *A. sarmentosa*. In 1981 Payoa et. al. reported the discovery of alkaloids in roots of *A. sarmentosa* by a chemical screening test. [13]

All organic constituents found in the Azima-genus plants are summarized in the Table 1.1:

Table 1.1 Chemical constituents found in plants of genus Azima

scientific name	plant part	substances	ref. No.
Azima sarmentosa	root	**alkaloid** (screening test)	13
Azima tetracantha	seed	**fatty acids:** - myristic, palmitic, stearic arachidic, behenic, oleic, linoleic and eicosenic acid -ricinoleic and cyclopropenoid fatty acid	10,11
	leaves	**dimeric piperadine alkaloid:** azimine (n=m=5) azacarpine (n=5,m=7) carpaine (n=m=7)	8
	leaves	**isorhamnetin 3-O-rutinoside**	12
	leaves & roots	**triterpenoids:** friedelin glutinol, lupeol, and β-sitosterol	9

Preliminarily biological screening test revealed the following interesting results : the hexane crude extract from the leaves was cytotoxic to brine shrimp LC_{50} 3.44 µg/ml (high activity) and interesting inhibitory activity on bacteria. Ethanolic crude extract of roots has medium cytotoxicity (LC_{50} 57.96 µg/ml) on brine shrimp and dichloromethane and ethyl acetate crude extracts of roots were significantly active on inhibition of rice growth.

Due to the interesting screening results and the lack of information of the chemical constituents and their biological activities, the research about *A. sarmentosa* should be continued. The research started by coarsely extracting specimens of the plant with suitable solvents, then isolating compounds from those crude extracts by chromatographic techniques. Structural elucidation of the isolated

compounds was deduced from spectroscopic evidences. Finally, biological tests of the isolated compounds were conducted.

1.3 The purpose of research

1. To isolate the organic compounds from *A. sarmentosa*.
2. To elucidate the structures of isolated compounds.
3. To study the bioactivities of isolated compounds.

Chapter II

Experimental

2.1 Plant material

The leaves and roots of *Azima sarmentosa* were collected in Samutsakorn Province during June 1998. The voucher specimens (003916) have been deposited in the Kasin Suvatabhandhu Herbarium, Department of Botany, Chulalongkorn University (BCU).

2.2 Chemicals

All solvents used in this thesis were purified by standard protocols, except for those which were reagent grade. Merck silica gel 60 G Art 7734 (70-230 mesh) was used as adsorbent for open-column chromatography, silica gel 7749 60 PF_{254} containing gypsum for chromatotron purification, and silica gel 7730 60 GF_{254} for preparative thin layer chromatography (PTLC). TLC spots were visualized with a UV lamp (254 and 365 nm) and with I_2 or 10% H_2SO_4 in ethanol.

2.3 General procedure

Melting points were determined with a Fisher-John melting point apparatus and are uncorrected. Chromatotron equipment, a Harrison Research Model 7924T was used for certain separations. Readymade TLCs, precoated with silica gel (Merck's, Kieselgel 60 G) on aluminium sheet, were used. Column chromatography was performed on silica gel 7734.

IR spectra were recorded on a Nicolet Impact 410 FT-IR. Mass spectra were obtained on Fissons MS800 mass spectrometer. ^1H and ^{13}C-NMR spectra

including 2D-NMR, were run in deuterated solvents ($CDCl_3$, acetone-d_6, or DMSO-d_6) with tetramethylsilane (TMS) as an internal reference on a Bruker Fourier Transformed Nuclear Magnetic Resonance Spectrometer, model AC-F200 and a Joel, model JNM-A500.

2.4 Extraction

Dried leaves (1.3 kg) and roots (10.80 kg) were coarsely ground and then soaked in ethanol (95%). The first extract was evaporated under reduced pressure to dryness, yielding the ethanolic crude extract. Then, the ethanolic crude extract was defatted by stirring with hexane and the hexane solution was evaporated under reduced pressure to give the hexane crude extract. The residue of this step was partitioned between dichloromethane and water, yielding the dichloromethane crude extract and a water soluble residue. The water soluble residue was partitioned between ethyl acetate, affording the ethyl acetate crude extract and the final water-soluble crude extract after concentration under reduced pressure.

The extraction of the roots was followed the same procedure as that of leaves. The procedure and results of the extraction are shown in Schemes 2.1 and 2.2.

Scheme 2.1 Extraction of the leaves of *A. sarmentosa*

Dried Ground Leaves of *A. sarmentosa* (1.3 Kg)

95% Ethanol

Ethanol Crude Extract (200 g, 15% w/w)   Marc (discarded)

Hex

Hex Crude Extract (38 g, 2.92% w/w)   Residue

$CH_2Cl_2 : H_2O$ (1: 1)

CH_2Cl_2 Crude Extract (8.58g , 0.66% w/w) H_2O Soluble Residue

EtOAc

EtOAc Crude Extract (2g, 0.15% w/w) H_2O Crude Extract
(120 g, 9.23% w/w)
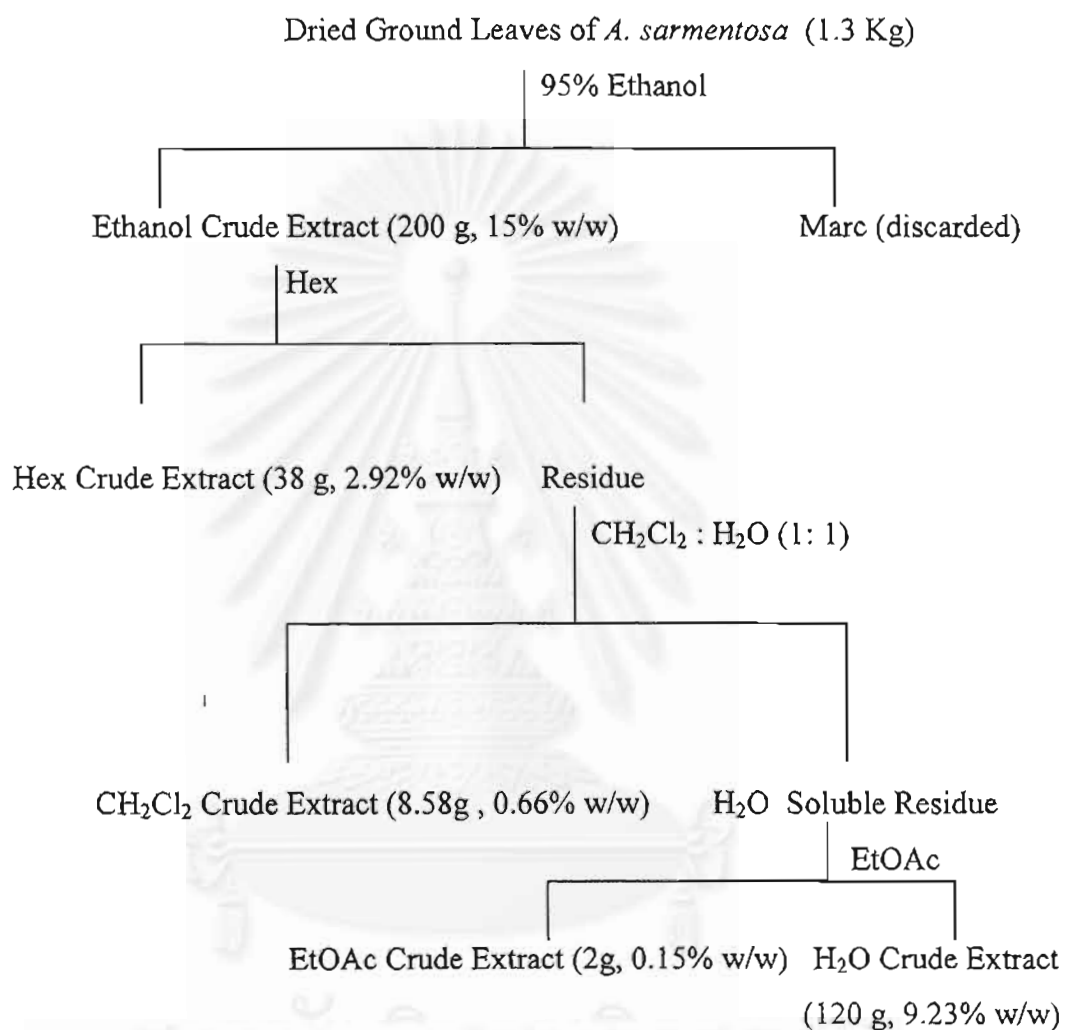
Scheme 2.2 Extraction of the roots of *A. sarmentosa*

Dried Ground Roots of *A. sarmentosa* (10.8 Kg)

95% Ethanol

Ethanol Crude Extract (801.2 g, 7.41% w/w)   Marc (discarded)

Hex

Hex Crude Extract (38 g, 0.35% w/w)   Residue

CH_2Cl_2 : H_2O (1: 1)

CH_2Cl_2 Crude Extract (40.0 g , 0.37% w/w) H_2O Soluble Residue

EtOAc

EtOAc Crude Extract (15.0 g, 0.14% w/w) H_2O Crude Extract

(~700 g, 6.48% w/w)
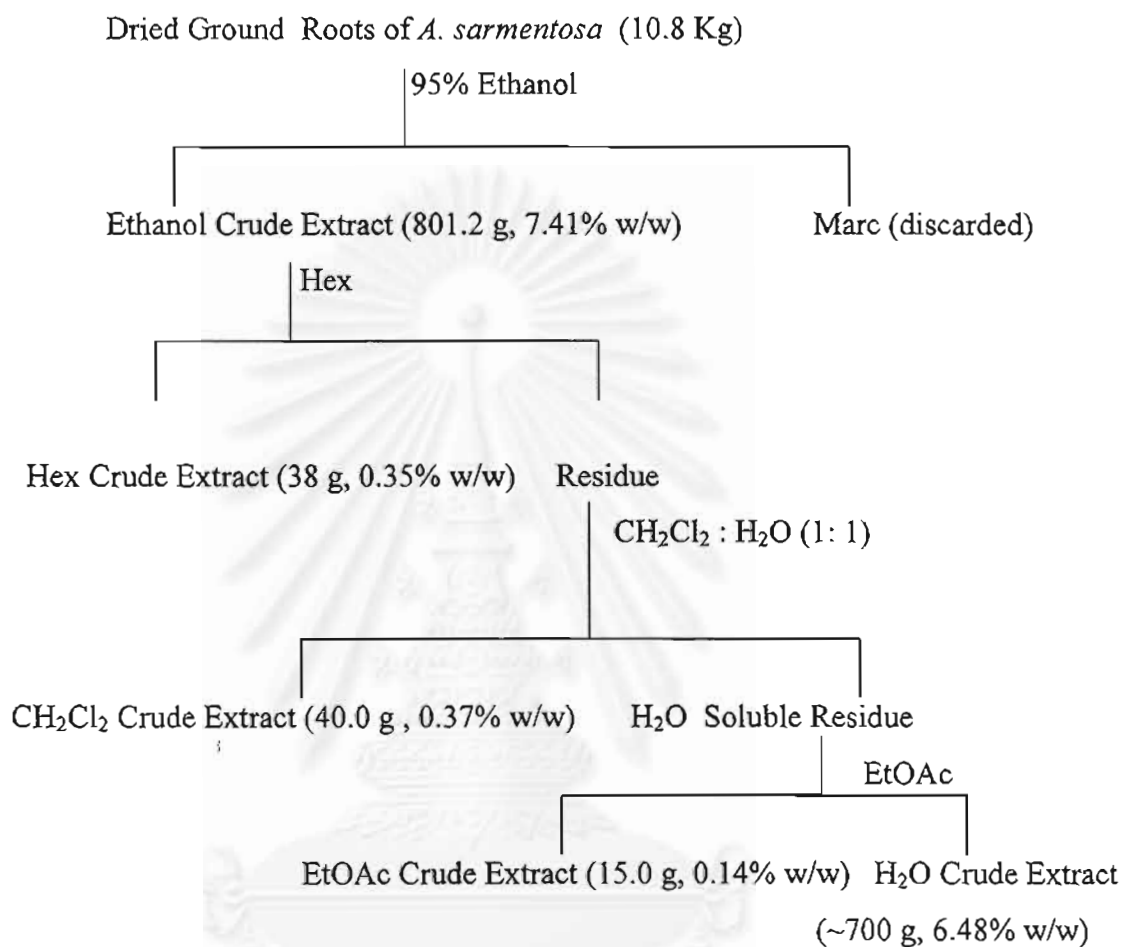
2.5 Separation and purification

The hexane crude extract of the leaves and the hexane, the dichloromethane and the ethyl acetate crude extracts of roots were separated by traditional open column chromatography. The extracts were dissolved in small amount of a suitable solvent and mixed with silica gel (1:1) to give the extract paste. The paste was evaporated to dryness under reduced pressure before being applied on the top of the column. The column was eluted by solvents in order of increasing polarity (hexane, dichloromethane, ethyl acetate, and methanol). Each fraction (about 500 ml) was collected, concentrated to small volume, and examined by TLC in order to group the fractions having the same components. The UV active fractions were further purified by column chromatography, chromatotron, or PTLC. Compounds isolated from each crude extract are shown in Schemes 2.3-2.6.

Scheme 2.3 Isolation procedure of the hexane crude extract of *A. sarmentosa* leaves

Hexane Crude Extract (38 g)

cc, SiO$_2$

100% Hex

Needle-white crystal

(**Compound 1**, 80 mg)

10% CH$_2$Cl$_2$-Hex

Needle-white Crystal

(**Compound 2**, 300 mg)

20% EtOAc-CH$_2$Cl$_2$

White Powder

(**Compound 3**, 15 mg)
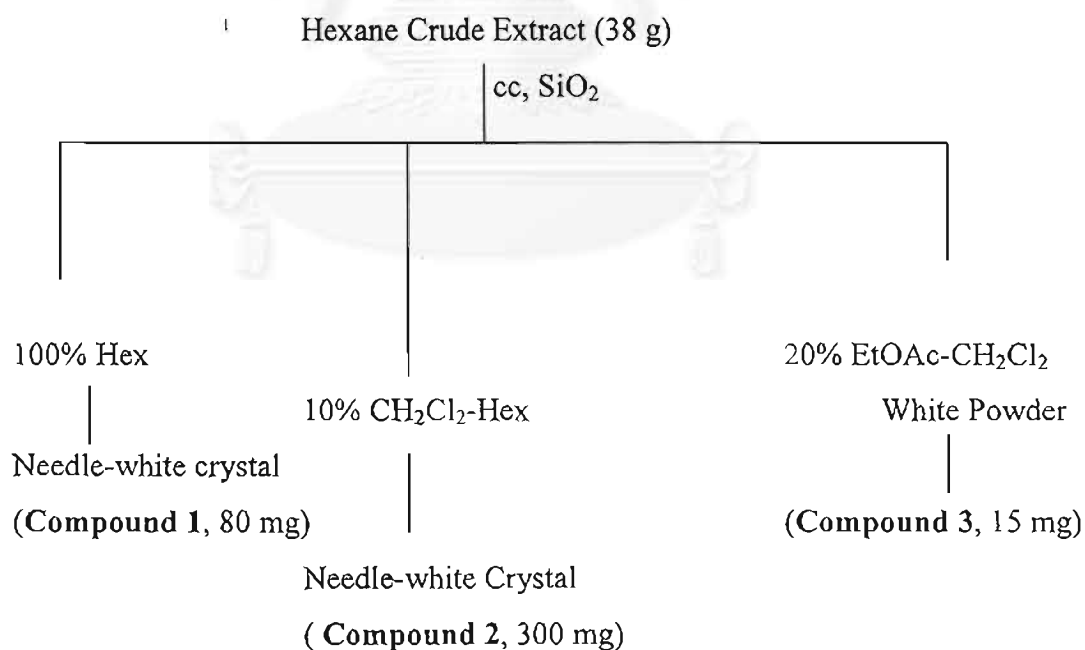
Scheme 2.4 Isolation procedure of the hexane crude extract of *A. sarmentosa* roots

Hexane Crude Extract (38 g)

cc, SiO$_2$

5% EtOAc-Hex

10% EtOAc-Hex

re, cc, SiO$_2$

chromatotron

Compound 4 (26.7 mg)

15% EtOAc-Hex 20% EtOAc-Hex

100% EtOAc

Compound 7 (20mg)

recrystallization

(CHCl$_3$: Hex, 1:1)

Compound 5 (350 mg)
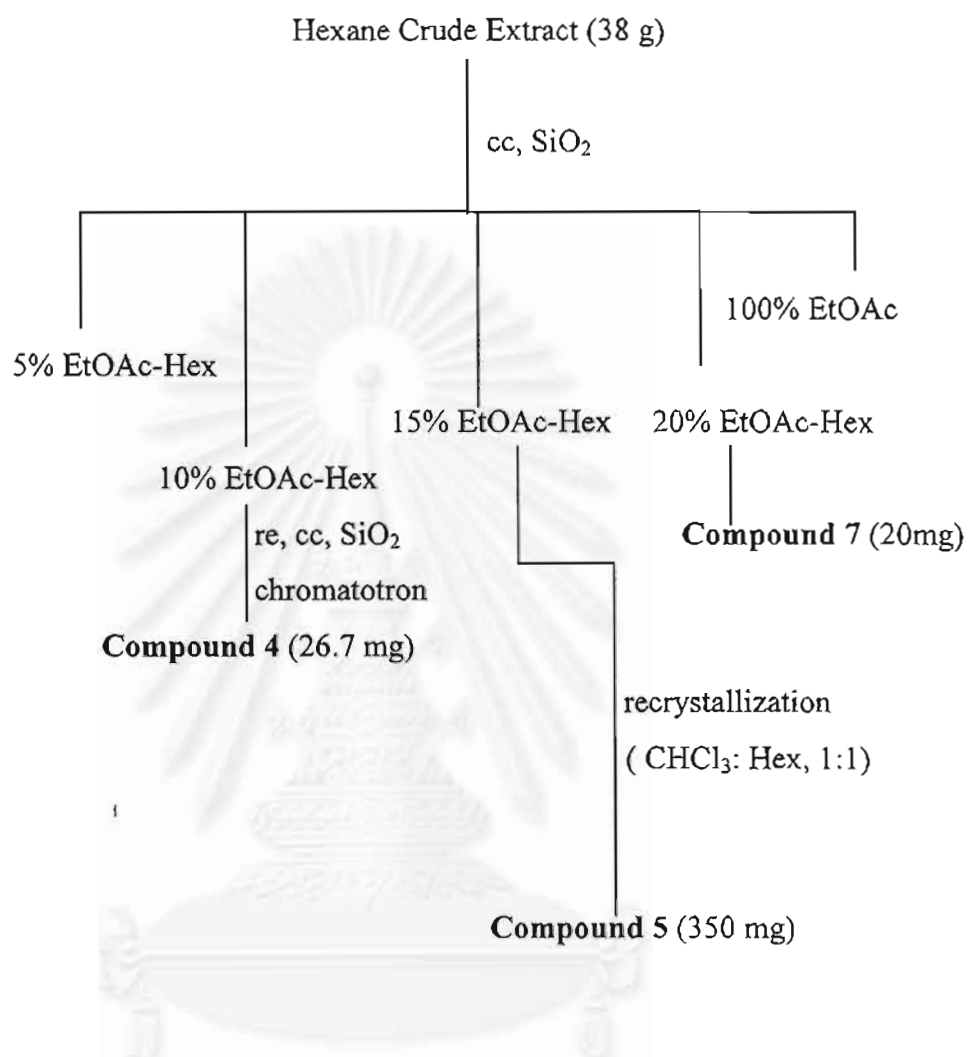
Scheme 2.5 Isolation procedure of the dichloromethane crude extract of

A. sarmentosa roots

CH_2Cl_2 Crude Extract (40 g)

cc, SiO_2

30% CH_2Cl_2-Hex   50% CH_2Cl_2-Hex   100% CH_2Cl_2 10% EtOAc-CH_2Cl_2 50% EtOAc-CH_2Cl_2

chromatotron   recrystallization

(MeOH: $CHCl_3$,

(Compound 6, 60 mg)   1:1)   **(Compound 10, 20 mg)**

(Compound 5, 45 mg)

column chromatography

(Compound 9, 108 mg)

(Compound 8, 80 mg)
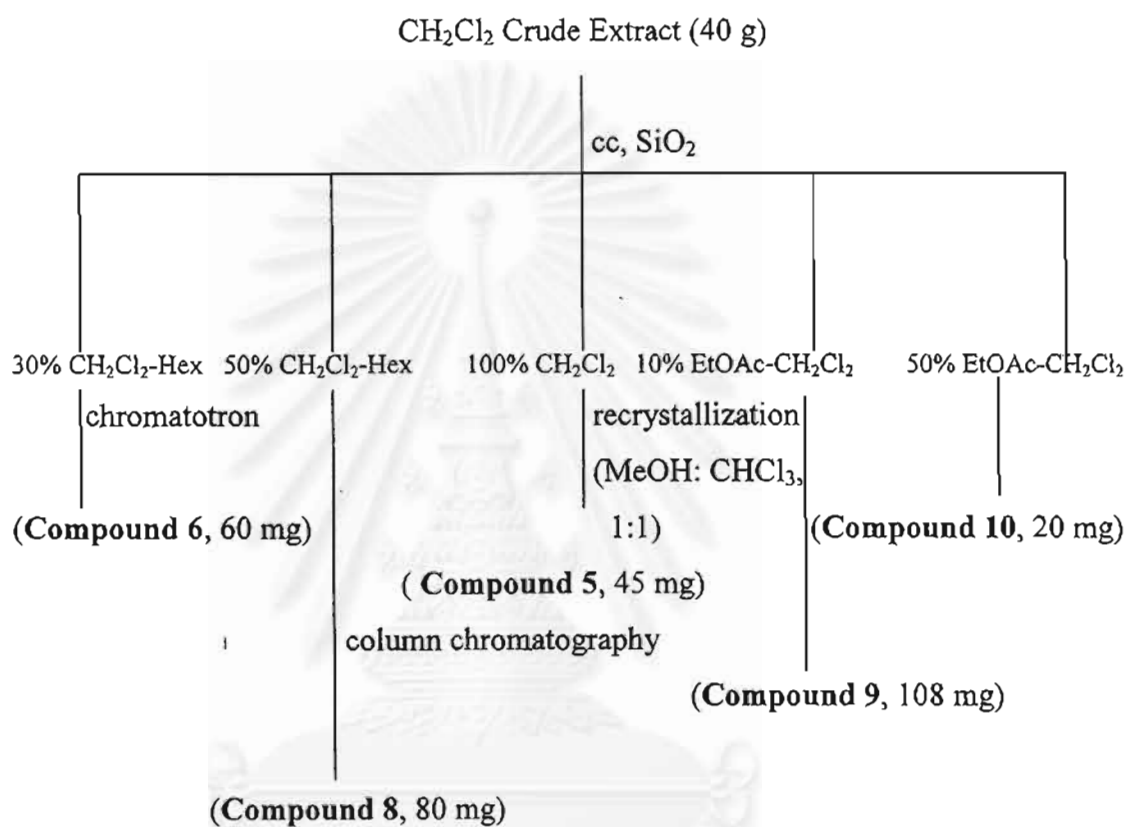
Scheme 2.6 Isolation procedure of the ethyl acetate crude extract of *A. sarmentosa* roots

EtOAc Crude Extract (15 g)

cc, SiO_2

100% CH_2Cl_2 20% EtOAc-CH_2Cl_2 10% MeOH-EtOAc

10% EtOAc-CH_2Cl_2

60% EtOAc-CH_2Cl_2

chromatotron   recrystalization

Compound 9 (25 mg)   (hot 100% MeOH)

Compound 10 (125 mg)
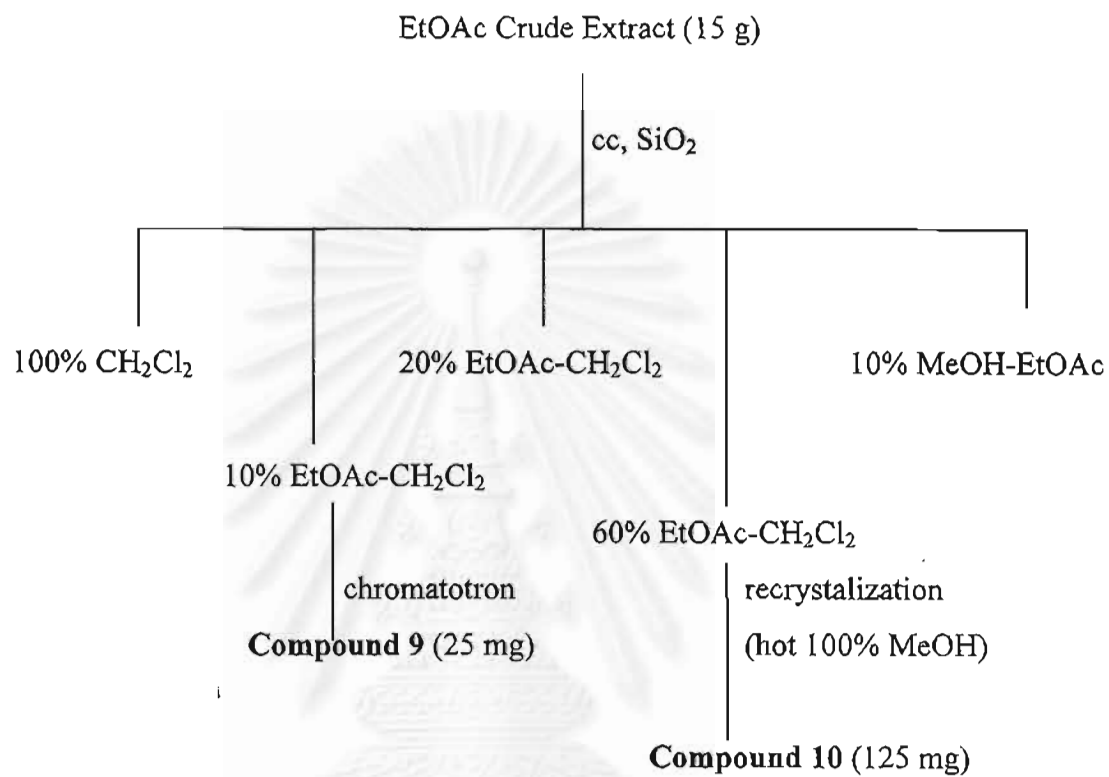
2.6 Bioassay experiments

Searching for bioactive compounds was the main goal of this research, therefore, the following bioassay experiments were undertaken.

2.6.1 Brine shrimp cytotoxic lethality test (BSCLT) [14]

Measuring the cytotoxicity to brine shrimp *Artemia salina* is such a basic bioassay. This method determines LC_{50} values in µg/ml which indicates the potential activity of the crude extracts and compounds. The advantages of this bioassay are rapidity, inexpensiveness and convenience for guiding phytochemical fractionation.

2.6.2 *In vitro* anti-tumor test

Several crude extracts of leaves and roots of *A. sarmentasa* Benth. were tested preliminarily by following the MTT assay. This method used five carcinoma cell lines: Human Gastric Carcinoma (BGC-823), Human Hepatocellular Carcinoma (Bel-7402), Human Nasopharyngeal (KB), Human Leukemia Carcinoma (HL-60), and Human Colon carcinoma (HCT-8). This experiment was conducted by researchers at Beijing Medical School, Beijing, China.

2.6.3 Plant growth inhibition test

The plant used as a monocotyledonal model in this experiment was *Oryza sativa* Linn. var. RD. 23 (rice). Seedlings of plants were cultured in cellulose powder which was readily mixed with solutions of the tested compounds. The controlled seedlings were also prepared in the same way. Seven days after germination, the length of roots and shoots of both treatment and controlled plants were measured. The percent of growth inhibition can be calculated by the formula below:

% Growth Inhibition =[1-(T/C)] X 100

where T and C are root and shoot length of treated and controlled seedlings, respectively.

2.6.4 Antioxidant activity [15]

It was determined by reduction of 2,2-diphenyl-1-pydrylhydrazyl or 2,2-Diphenyl-1- (2,4,6-trinitrophenyl) hydrazyl; DPPH radical. TLC autographs assay: after developing and drying, TLC plates were sprayed with a 0.2% DPPH solution in MeOH. The plates were examined at 30 min. after spraying. Active compounds appeared as yellow spots against a purple background.

Antioxidant was also determined by bleaching of β-carotene. TLC autographic assay: after developing and drying, TLC plates were sprayed with a β-carotene solution in chloroform (0.2 mg/ml). The plates were exposed to 254 nm UV light for 20 min before examination. β-carotene undergoes bleaching except in plates where antioxidative substances prevented the degradation. Active compounds appeared as orange spots against a white background.

Chapter III
Results and Discussion

3.1 Preliminary screening results of the crude extracts

3.1.1 Brine shrimp cytotoxic lethality test (BSCLT)

This primary screening test was assisted by staff of the Department of Biology, Faculty of Science, Chulalongkorn University. BSCLT results of extracts are shown below:

Table 3.1 Brine shrimp cytotoxic lethality test of various crude extracts of leaves

crude extracts	LC_{50} ($\mu g/ml$)	activity
ethanol	25.58	medium activity
hexane	3.44	high activity
dichloromethane*	-	-
ethyl acetate	199.53	low activity
water*	-	-

* These crude extracts have not been tested.

The hexane crude extract showed significant toxicity to brine shrimp, so this crude extract of the leaves of *A. sarmentosa* was selected for further isolation.

Table 3.2 Brine shrimp cytotoxic lethality of crude extract of roots

Crude extracts	LC_{50} ($\mu g/ml$)	Bioactivity
ethanol	57.96	medium activity

High activity ($LC_{50} < 10$ $\mu g/ml$)

Medium activity ($LC_{50} < 100$ $\mu g/ml$)

Low activity ($LC_{50} < 1000$ $\mu g/ml$)

No activity ($LC_{50} > 1000$ $\mu g/ml$)

3.1.2 Antibacterial Activity

To preliminarily screen of the crude extracts, antibacterial bioassay was selected. There were many types of bacterias tested: *E.coli*, *B.cereus*, *S.aureus*, *S.derby*, *E.coli* 0157;H7, *L.monocytogenes* and Flat sour spoilage)

After 24 hours of incubation, the diameter of the clear zone was measured. A diameter of inhibition zone larger than 10 mm was estimated to be high inhibition (++), 7-10 mm as weak inhibition (+), less than 7 mm as inactive(-). The results are shown in Table 3.3.

Table 3.3 Antibacterial activity of crude extracts of the leaves against several kinds of bacteria at a dosage of 300 µg/disc

crude extracts	Antibacterial estimation of Crude extracts against						
	1	2	3	4	5	6	7
ethanol	++	++	++	++	++	++	+
hexane	++	++	++	++	++	+	+
dichloromethane	-	-	-	-	-	-	-
ethyl acetate	-	-	-	-	-	-	-
water	++	++	++	+	++	+	+

(1 = *E.coli*, 2 = *B.cereus*, 3 = *S.aureus*, 4= *S.derby*, 5 = *E.coli* 0157;H7, 6= *L.monocytogenes*, 7 = Flat sour spoilage)

It was concluded that the ethanol, hexane and water crude extracts gave promising results as antibacterial because they had inhibitory activities against various types of bacteria.

3.1.3 Plant growth inhibition test

The plant used in this experiment was *Oryza sativa* var. RD 23 which was selected as a model of monocotyledonal plants because the seedling's root and shoots are stable and has the same trend. The results are shown in Table 3.4.

Table 3.4 Effect of some root crude extracts on growth of *Oryza sativa* var. RD.23 at various concentrations

crude extracts	% Inhibition							
	root				shoot			
	concentration (ppm)				concentration (ppm)			
	10	100	1000	10000	10	100	1000	10000
ethanol	-4.04	4.99	32.07	17.34	-8.39	-17.42	39.35	16.13
dichloromethane	13.54	14.01	49.41	89.07	1.94	3.23	3.23	30.97
ethyl acetate	7.13	12.35	22.57	87.65	-14.19	-24.52	25.61	15.48
water	-23.52	21.85	33.73	-22.09	-22.09	9.68	34.84	32.90

note: - Hexane crude extract has not been tested.

- % Inhibition less than zero is of % promotion.

Considering the data from rice-growth inhibition test, it can be found that in the dichloromethane and the ethyl acetate crude extracts, when the concentration is increased, the inhibitory activity is also increased. Of this result, it implied that when we applied these crude extracts to be used as plant growth inhibitor, we can change the concentration of crude extracts for suitable activity depending on plant we want to treat. Therefore, the dichloromethane and the ethyl acetate crude extracts exhibited relatively interesting activity.

Table 3.5 Effect of some leaf crude extracts on growth of *Oryza sativa* var. RD.23 at various concentrations

crude extracts	% Inhibition							
	root				shoot			
	concentration (ppm)				concentration (ppm)			
	10	100	1000	10000	10	100	1000	10000
ethanol	14.49	5.23	4.75	36.82	37.42	22.58	-7.74	27.74
dichloromethane	13.54	14.01	49.41	89.07	1.94	3.23	3.23	30.97
water	-23.52	21.85	33.73	-22.09	-22.09	9.68	34.84	32.90

3.1.4 *In vitro* anti-tumor result

   In vitro anti-tumor bioassay was one of the selected primary screening. All crude extracts were tested and the result is shown in the Tables 3.6 and 3.7.

Table 3.6 Primary screen for HCT-8 and Bel-7402

crude extract	Concentration	HCT-8		Bel-7402	
		inhibition	estimation	inhibition	estimation
root	(µg/ml)	(%)		(%)	
ethanol	1	-0.65		-7.89	
	10	3.84	-	-7.89	-
	100	40.61		15.46	
hexane	1	3.41		-4.79	
	10	11.33	-	2.46	-
	100	22.00		43.62	
dichloromethane	1	-0.86		-2.20	
	10	16.48	-	-5.17	-
	100	23.38		22.79	
ethyl acetate	1	5.49		-4.48	
	10	10.23	-	1.76	-
	100	24.89		21.40	
water	1	1.72		-4.22	
	10	12.06	-	-3.91	-
	100	26.07		20.58	

Table 3.6 (cont.)

Leaf					
ethanol	1	4.41		-2.77	
	10	20.04	-	-2.39	-
	100	37.50		42.23	
hexane	1	8.83		-5.23	
	10	17.45	-	-1.95	-
	100	31.03		39.77	
dichloromethane	1	9.37		-8.20	
	10	15.84	-	-2.52	-
	100	29.20		36.80	
water	1	-4.41		0.84	
	10	12.71	-	6.84	-
	100	17.45		21.15	

Table 3.7 Primary screen for HL-60 and BGC-823

		HL-60		BGC-823	
crude extract	Concentration	inhibition	estimation	inhibition	estimation
root	(μg/ml)	(%)		(%)	
ethanol	1	-41.74		5.20	
	10	-47.43	-	0.28	-
	100	31.20		20.95	
hexane	1	3.41		10.75	
	10	11.33	-	8.08	-
	100	22.00		33.96	
dichloromethane	1	-74.20		29.32	
	10	-37.44	-	16.24	-
	100	34.95		33.12	
ethyl acetate	1	-39.83		10.75	
	10	-18.08	-	32.06	-
	100	2.91		33.47	
water	1	-70.59		34.88	
	10	-53.95	-	25.17	-
	100	31.06		25.17	

Table 3.7 (cont.)

Leaf					
ethanol	1	-4.82		37.97	
	10	26.83	-	33.55	-
	100	48.16		35.27	
hexane	1	-50.32		30.23	
	10	-9.06	+	24.89	-
	100	85.93		46.41	
dichloromethane	1	-17.20		37.76	
	10	-6.75	+	27.63	-
	100	70.39		33.68	
water	1	-30.43		14.34	
	10	-6.66	-	8.60	-
	100	13.50		28.68	

HCT-8: Human Colon Carcinomar   Bel-7402: Hepatocellular Carcinoma

HL-60 : Human Leukemia Carcinoma BGC-823: Human Gastric Carcinoma

The data in Tables 3.6 ad 3.7 showed no significant activity for the crude extracts in this bioassay.

3.2 Separation of crude extracts of the leaves of *A. sarmentosa*

The hexane crude extract, 38 g was selected for further separation because of its high activity on brine shrimp lethality test. The result of separation is shown in table below.

Table 3.8 The separation of the hexane crude extract of the leaves

Fr.No	eluent	fr.No.	remark	weight (g)
1	100% Hex	1	dark viscous liquid	2.79
2	100% Hex	2	dark viscous liquid with yellow oil	0.71
3	100% Hex	3	yellow oil	0.35
4	100% Hex	4	dark viscous liquid with yellow oil	0.84
5	100% Hex	5	white solid	0.65
6	100% Hex	6-7	white needle crystal	0.38
7	10% CH_2Cl_2-Hex	8-11	dark viscous liquid	1.23
8	10% CH_2Cl_2-Hex	12-17	white powder with yellow oil	0.18
9	10% CH_2Cl_2-Hex	18-24	white crystal	7.46
10	10, 20 and 50% CH_2Cl_2- Hex	25-37	white crystal	0.61
11	50% CH_2Cl_2-Hex	38-47	dark viscous liquid with white crystal	0.98
12	50% CH_2Cl_2-Hex	48-53	green residue	0.28
13	70% CH_2Cl_2-Hex	54-65	brown viscous liquid	1.19
14	70 and 80% CH_2Cl_2-Hex	66-76	brown viscous liquid	0.54
15	80% CH_2Cl_2-Hex	77-85	brown viscous liquid	0.85
16	80% CH_2Cl_2-Hex and 100% CH_2Cl_2	86-102	brown viscous liquid	0.76
17	100% CH_2Cl_2 and 10% EtOAc-CH_2Cl_2	103-122	brown viscous liquid	3.24
18	10 and 20% EtOAc-CH_2Cl_2	123-130	brown viscous liquid	1.43
19	20% EtOAc-CH_2Cl_2	131-134	brown viscous liquid	0.34
20	20% EtOAc-CH_2Cl_2	135-140	brown viscous liquid with white powder	0.77
21	20% and 50% EtOAc-CH_2Cl_2	141-170	brown viscous liquid	1.84
22	70% EtOAc-CH_2Cl_2 and 100% EtOAc	171-186	brown viscous liquid	1.67
23	100% EtOAc and 10% MeOH-EtOAc	187-195	brown viscous liquid	2.66
24	10% MeOH-EtOAc and 100% MeOH	196-200	brown viscous liquid	1.81

3.3 Separation of crude extracts of the roots of *A.sarmentosa*

3.3.1 Separation of the hexane crude extract

40 g of the hexane crude extract was preadsorbed on silica gel prior to application to the top of the column, then eluted with solvents in order of polarity. The results of the separation are shown in the table below.

Table 3.9 The separation result of hexane crude extract of the roots of *A. sarmentosa*

Fr.No.	eluent	fr.No.	remark	weight (g)
1	5% EtOAc-Hex	1-3	pale-yellow liquid	3.98
2	5% EtOAc-Hex	4-5	orange viscous liquid with white needle solid	0.52
3	5% and 10% EtOAc-Hex	6-15	solid orange viscous liquid with white needle solid	3.76
4	10% EtOAc-Hex	16-20	pale-yellow wax	2.38
5	15% EtOAc-Hex	21-23	orange wax with white solid	4.02
6	15% EtOAc-Hex	24-30	solid orange viscous liquid with white solid	5.87
7	15% EtOAc-Hex	31-35	brown viscous liquid	1.85
8	20% EtOAc-Hex	36-41	brown viscous liquid	2.34
9	20% EtOAc-Hex	42-47	brown viscous liquid	0.83
10	20 and 30% EtOAc-Hex	48-65	brown viscous liquid	1.12
11	30% EtOAc-Hex	66-80	brown residue	1.05
12	40% EtOAc-Hex	81-90	brown residue	0.72
13	60 and 80% EtOAc-Hex	91-110	brown residue	1.54
14	100% EtOAc	111-120	brown residue	1.87

3.3.2 Separation of the dichloromethane crude extract

40 g of the dichloromethane crude extract was separated by open column chromatography with silica gel as an adsorbent. The results of the separation are shown below.

Table 3.10 The separation of the dichloromethane crude extract of the roots of *A. sarmentosa*

Fr.No.	eluent	fr.No.	remark	weight (g)
1	30% CH_2Cl_2-Hex	1-19	orange oil	
2	50% CH_2Cl_2-Hex	20-29	orange oil with white needle solid	1.33
3	50 and 70% CH_2Cl_2-Hex	30-42	orange oil with white needle solid	
4	70% CH_2Cl_2-Hex	43-61	brown viscous liquid	1.50
5	100%CH_2Cl_2	62-65	brown viscous liquid with white solid	0.50
6	100%CH_2Cl_2	66-85	brown viscous liquid with white solid	5.29
7	100%CH_2Cl_2 and 10% EtOAc-CH_2Cl_2	86-100	brown viscous liquid	2.84
8	10% EtOAc-CH_2Cl_2	101-110	brown viscous liquid	4.35
9	20 and 50% EtOAc-CH_2Cl_2	111-145	brown viscous liquid	2.91
10	50% EtOAc-CH_2Cl_2	146-159	brown viscous liquid with white powder	3.05
11	70% EtOAc-CH_2Cl_2	160-177	brown viscous liquid	0.52
12	100% EtOAc	178-199	brown viscous liquid	5.66
13	10% MeOH-EtOAc	200-210	brown viscous liquid	2.40
14	10% MeOH-EtOAc	211-220	brown viscous liquid	7.25

3.3.3 Separation of the ethyl acetate crude extract

25 g of ethyl acetate crude extract was separated by column chromatography. The results are shown in Table 3.11.

Table 3.11 The separation of the ethyl acetate crude extract of the roots of *A. sarmentosa*

Fr.No	eluent	fr.No.	remark	weight (g)
1	100% CH$_2$Cl$_2$	1-2	brown viscous liquid	0.21
2	100% CH$_2$Cl$_2$	3	brown viscous liquid	0.34
3	10% EtOAc-CH$_2$Cl$_2$	4-13	brown viscous liquid	0.63
4	10% EtOAc-CH$_2$Cl$_2$	14-17	brown viscous liquid	2.31
5	10% EtOAc-CH$_2$Cl$_2$	18-20	brown viscous liquid with white solid	1.85
6	20 and 40% EtOAc-CH$_2$Cl$_2$	21-37	brown viscous liquid	0.98
7	60% EtOAc-CH$_2$Cl$_2$	38-45	brown viscous liquid	2.46
8	60 and 80% EtOAc-CH$_2$Cl$_2$	46-53	brown viscous liquid	2.31
9	80% EtOAc-CH$_2$Cl$_2$ and 100% EtOAc	54-74	brown residue	2.87
10	10% MeOH-EtOAc	75-85	brown residue	1.34

3.4 Physical properties and structural elucidation of isolated compounds

3.4.1 Physical properties and structural elucidation of Compound 1

Compound 1 was obtained from Fr.No. 5 of the hexane crude extract when eluted with 100% hexane. After recrystallization from chloroform and hexane (1:1), white needle crystals of Compound 1 were obtained (80 mg, 0.21 % w/w of hexane crude extract) m.p. 245-246 ^{0}C, R$_f$ 0.65 in 100% chloroform. This compound gave a purple color with Liebermann-Burchard's and also decolorized Br$_2$ in CCl$_4$. These reactions are typical of a triterpenoidal skeleton including unsaturation in its structure.

The IR spectrum (Fig.3.1) assigned in Table 3.10 showed the important characteristic absorption band of a carbonyl group at 1708 cm^{-1} and additional bands due to a trisubstituted olefinic moiety at 3040 and 815 cm^{-1}.

Table 3.12 Some important IR absorption bands of Compound 1

frequency (cm^{-1})	band type	assignment
3040	w	C-H stretching of alkene
2937, 2882	s	C-H stretching of –CH$_3$
1708	s	C-O stretching of carbonyl
1462, 1378	m	C-H bending of –CH$_3$ and –CH$_2$
815	w	C-H out of plane bending of trisub. olefin

The ^{1}H NMR spectrum (Fig. 3.2) showed an important signal at 5.51 ppm (1H, dd, J = 6.0, 6.2 Hz) attributed to the signal of an olefinic proton but no signals of aromatic protons. The ^{13}C NMR spectrum (Fig. 3.3) showed the olefinic carbon signals positioned at 157.6 and 117.2 ppm and a carbonyl carbon at 217.6 ppm.

In the mass spectrum (Fig. 3.4), the parent ion peak at m/z 425 (M+1)$^+$ could be seen. The molecular formula of this compound was proposed to be $C_{30}H_{48}O$ (MW. 424.39). Other ions, which are the characteristic fragmentation of taraxerane triterpenoid were displayed in Scheme 3.1.

To confirm the structure of this compound, the ^{13}C NMR chemical shifts were compared with those reported[16] (Table 3.13). A close correspondance was found.
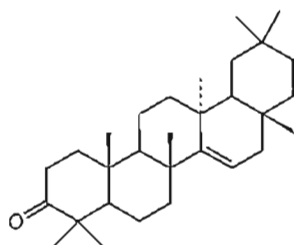
Table 3.13 ^{13}C NMR chemical shifts of taraxerone compared to those of Compound 1

carbon No.	taraxerone	Compound 1
1	38.4	38.3
2	34.1	34.1
3	217.3	217.6
4	47.6	47.6
5	55.8	55.8

Table 3.13 (cont.)

Carbon No.	Taraxerone	Compound 1
6	20.0	19.9
7	35.2	35.1
8	38.9	38.9
9	48.7	48.7
10	37.6	37.5
11	17.5	17.4
12	35.8	35.8
13	37.7	37.7
14	157.6	157.6
15	117.2	117.2
16	36.7	36.6
17	37.7	37.7
18	48.8	48.7
19	40.7	40.6
20	28.8	28.8
21	33.6	33.5
22	33.1	33.1
23	26.2	26.1
24	21.5	21.5
25	14.8	14.8
26	29.9	29.8
27	25.6	25.6
28	29.9	29.6
29	33.4	33.3
30	21.4	21.3

From the data above, it can be concluded that Compound 1 was taraxerone and the structure of Compound 1 can be shown below:

Structure of Compound 1

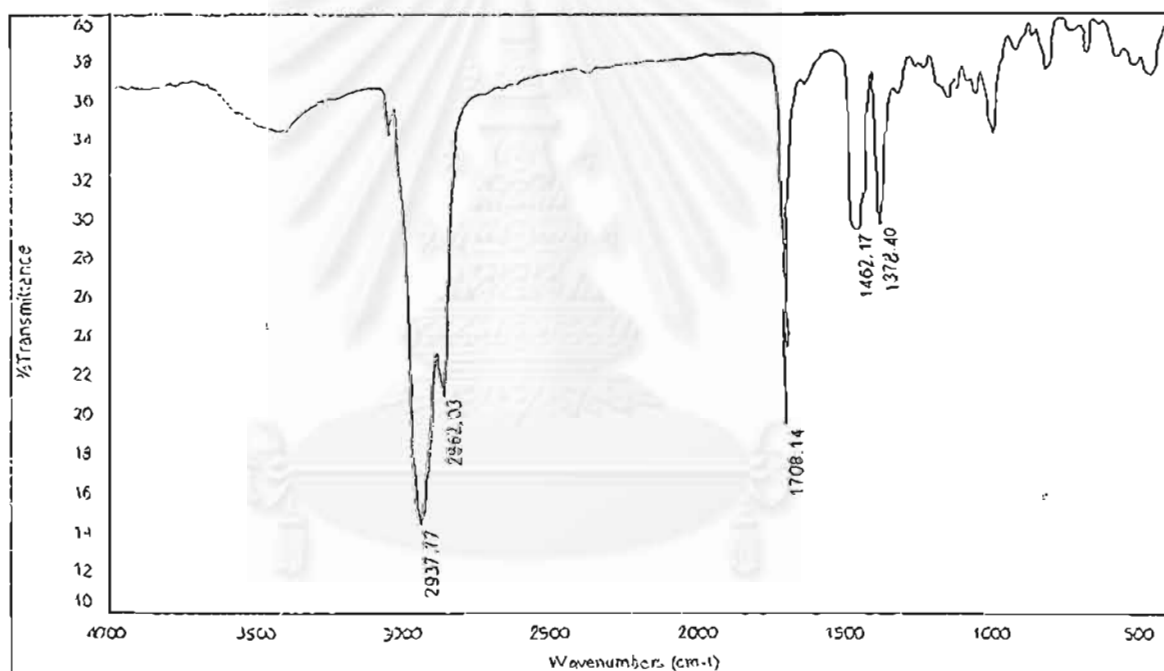**Scheme 3.1** Possible mass fragmentation of Compound **1**

m/z 424

C-8 migration

m/z 300

C-13 migration

cleavage 11-12, and 8-14 bonds

m/z 204

-CH3
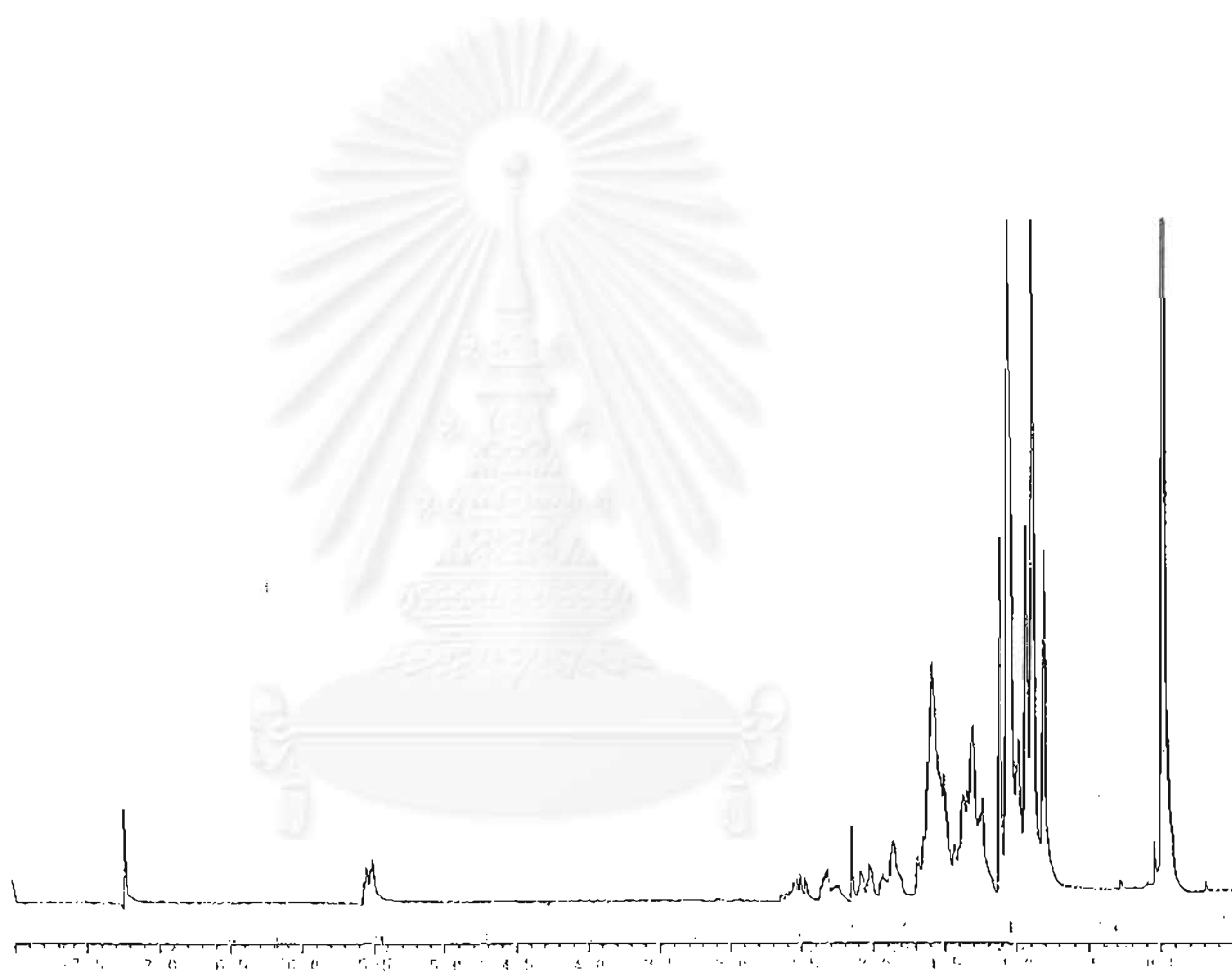
m/z 189
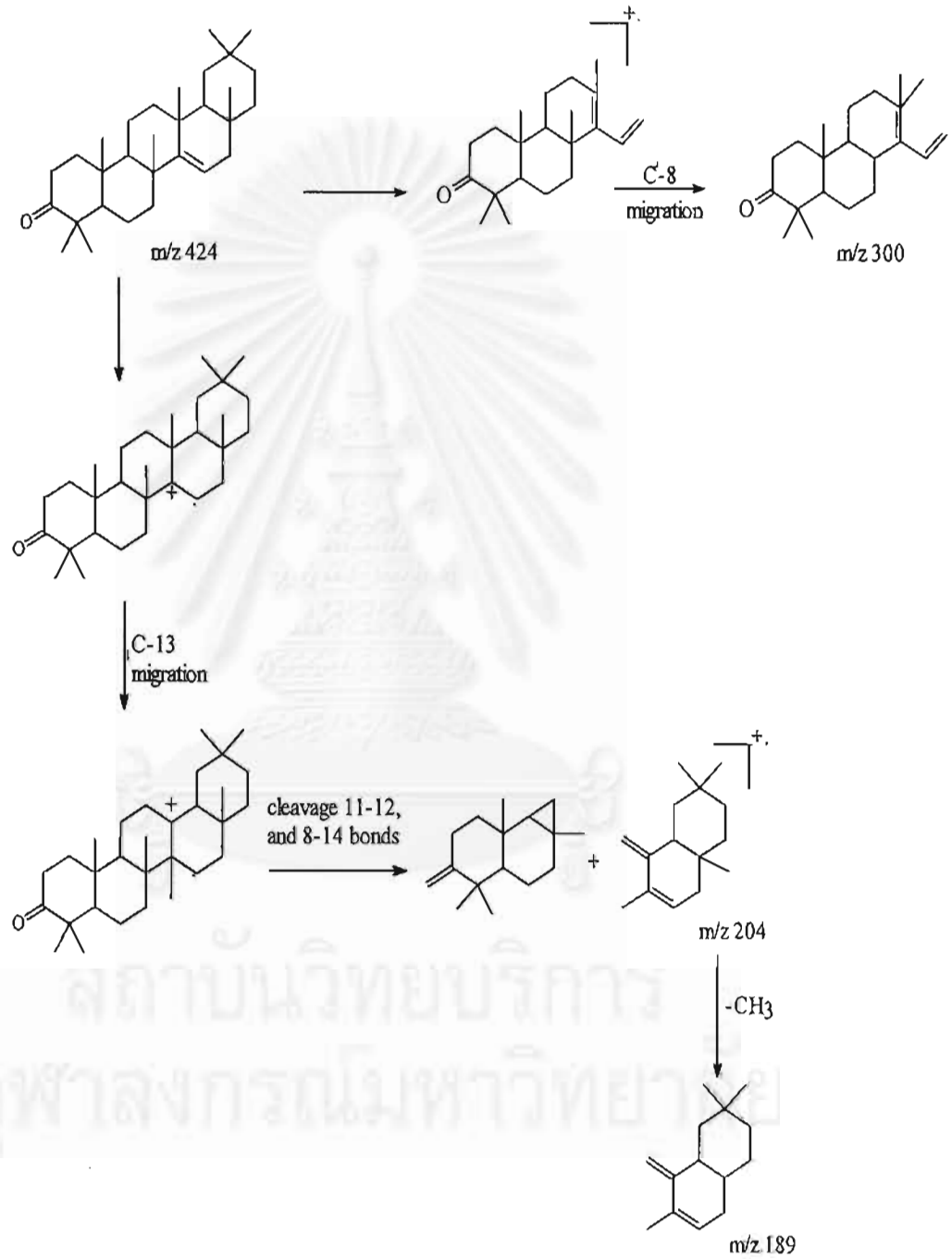
Fig. 3.1 The IR spectrum of Compound 1

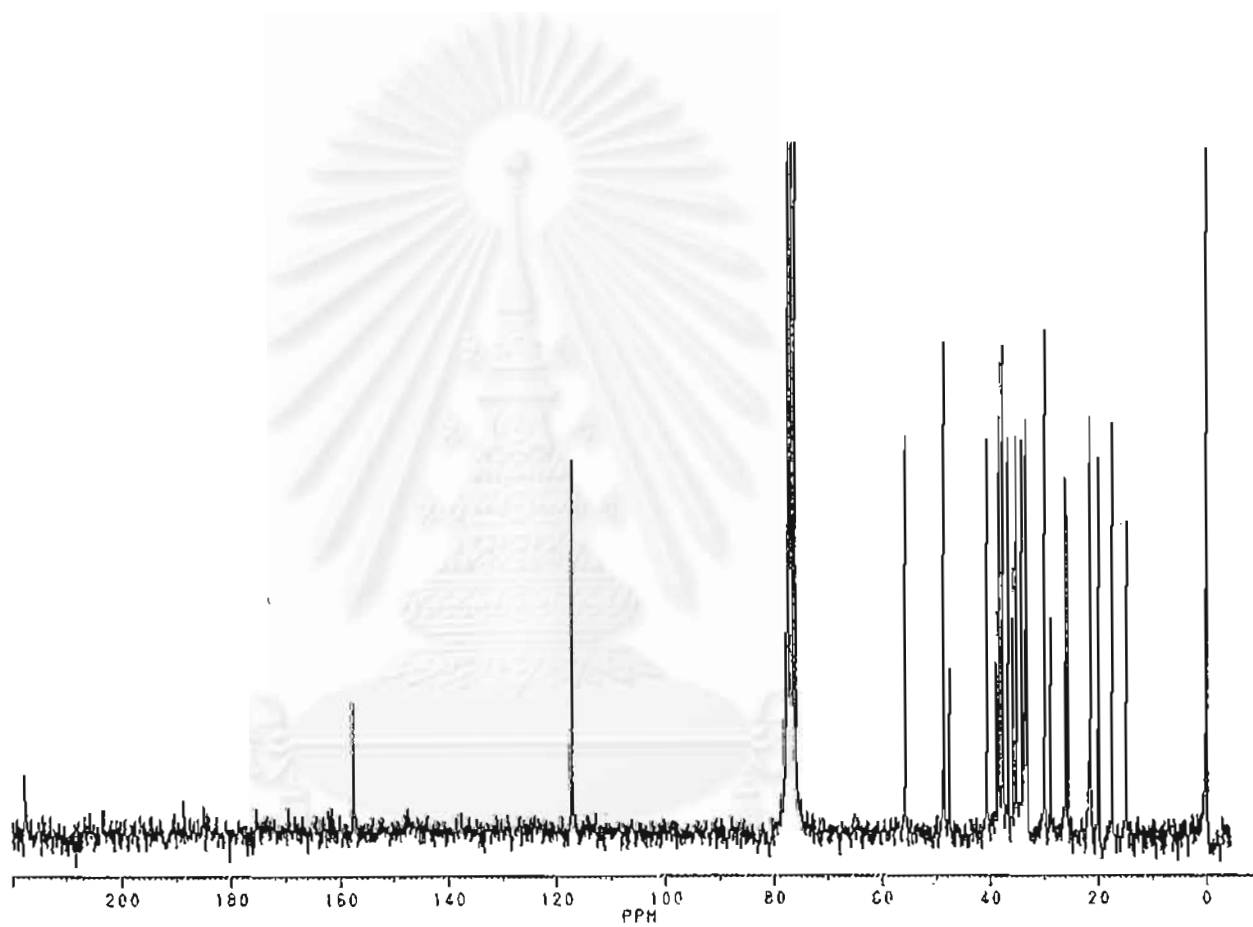Fig. 3.2 The ^{1}H NMR spectrum of Compound **1**

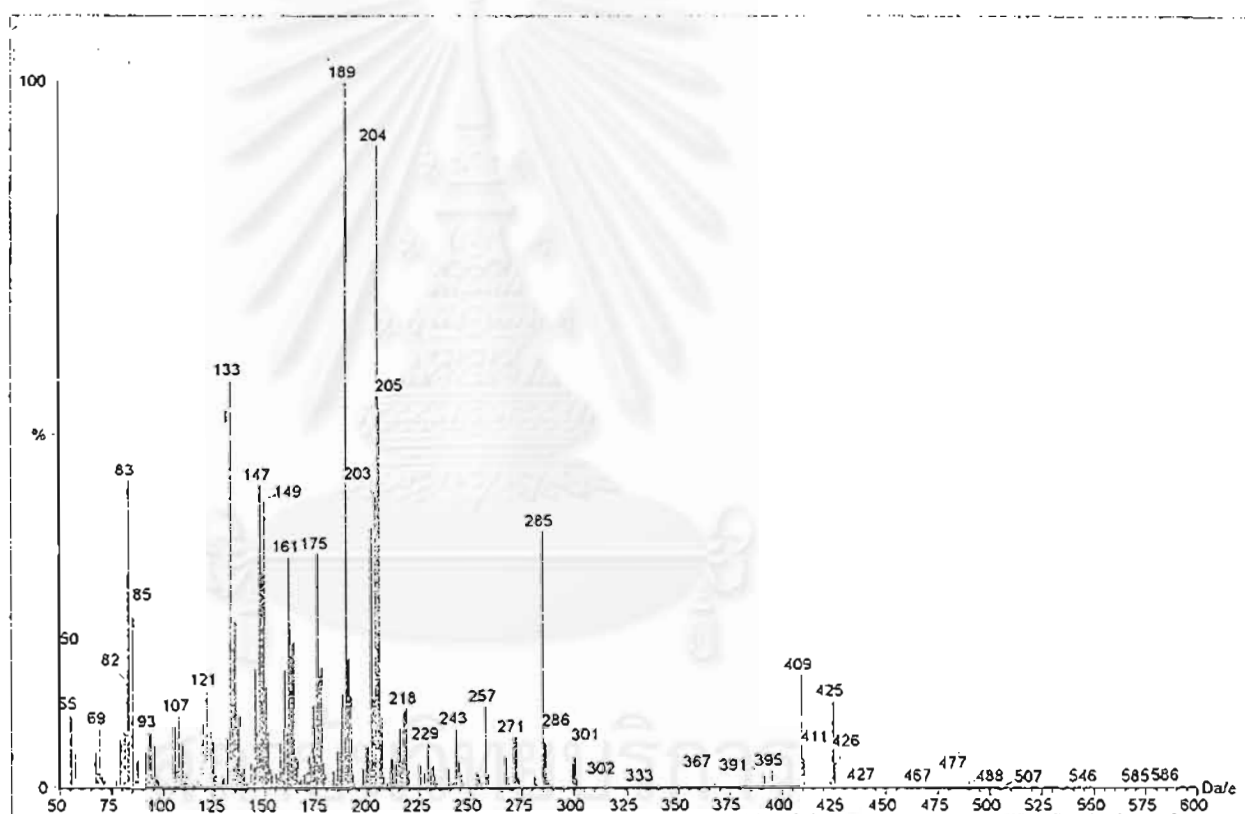**Fig. 3.3** The ^{13}C NMR spectrum of Compound 1

Fig. 3.4 The mass spectrum of Compound 1

3.4.2 Physical properties and structural elucidation of Compound 2

After elution with 10% dichloromethane-hexane, Compound 2 was obtained as white powder. Recrystallization from chloroform-hexane (1:1) gave white-needle crystal, 300 mg (0.79 % w/w) m.p. 280-284 ^{0}C, R_f 0.45 in 100% chloroform solvent system. This compound showed a violet color with Liebermann-Burchard's reagent which indicated a triterpenoid-containing compound.

The IR spectrum (Fig. 3.5), assigned in Table 3.12, showed the characteristic absorption band belonging to –OH at 3490, 1040 and 1005 cm^{-1} and additional bands of a characteristic trisubstituted olefinic moiety at 3050, 1645 and 815 cm^{-1}.[17]

Table3.14 The IR absorbtion band assignment of Compound 2

frequency(cm^{-1})	band type	tentative assignment
3490	m	O-H stretching vibration
3050	w	C-H stretching vibration of $R_1R_2C=CR_3H$
2995, 2940	s	C-H stretching vibration of –CH$_3$
2870, 2850	s	C-H stretching vibration of –CH$_2$-
1645	w	C=C stretching vibration
1475,1385	s	C-H bending vibration of –CH$_3$ and –CH$_2$-
1040,1005	s	C-O stretching vibration of 3β-OH
815	w	C-H out of plane bending of trisub.olefin

The ^{1}H-NMR spectrum (Fig. 3.6) showed a significant signal at 5.53 ppm (1H, dd, J=3.43, 7.55 Hz) attributable to the signal of an olefinic proton[18] and at 3.21 ppm (1H, t, J = 6.93 Hz) consistent with the signal of a proton on a carbon attached to an oxygen atom.

The molecular formula of this compound was expected to be $C_{30}H_{50}O$ (MW 426.39) based on the mass spectrum (Fig.3.8) which exhibited the parent ion peak at m/z 426.0 (calcd. for $C_{30}H_{50}O$: MW. 426.39) and other fragmentation ion

peaks at m/z 411.0 (M^+-CH_3), 408.0 (M^+-H_2O), 393.0 (M^+-CH_3-H_2O), 302 (RDA), 287.0 (RDA-CH_3), 269.0 (RDA-CH_3-H_2O), 204.0(cleavage of 11-12 and 8-14 bonds) and 189.0 (204-CH_3). The series of fragmentation ion pattern at m/z 302.0, 269.0, 204.0, and 189.0 implied that this triterpenoid should be a member of the group of taraxerane triterpenoidal compounds.[19] The possible mass fragmentation pattern of Compound 2 is illustrated in Scheme 3.2.

By means of TLC and spectral comparison, the structure Compound 2 can be proposed as the triterpenoid, taraxerol. The ^{13}C-NMR chemical shift assignment of Compound 2 closely corresponded to that of taraxerol.[20] Chemical shifts of both Compound 2 and taraxerol are presented in Table 3.13.

Table 3.15 Comparison of ^{13}C NMR chemical shifts of taraxerol and Compound 2

carbon position	taraxerol	Compound 2
1	158.2	158.1
2	117.0	116.9
3	79.1	79.1
4	55.6	55.5
5	49.4	49.3
6	48.9	48.8
7	41.4	41.3
8	39.1	39.0
9	38.8	38.7
10	38.7	38.7
11	37.8	38.0
12	37.8	37.7
13	37.6	37.5
14	36.8	36.7
15	35.8	35.8
16	35.2	35.1
17	33.8	33.7
18	33.4	33.3
19	33.2	33.1

Table 3.15 (cont.)

carbon position	taraxerol	Compound 2
20	29.9	29.9
21	29.7	29.8
22	28.8	28.8
23	28.0	28.0
24	27.3	27.1
25	26.0	25.9
26	21.1	21.3
27	18.9	18.8
28	17.6	17.5
29	15.5	15.4
30	15.5	15.4

According to the physical and chemical properties of this compound, the major component of this fraction had to be taraxerol. The structure of this triterpenoid is shown below.
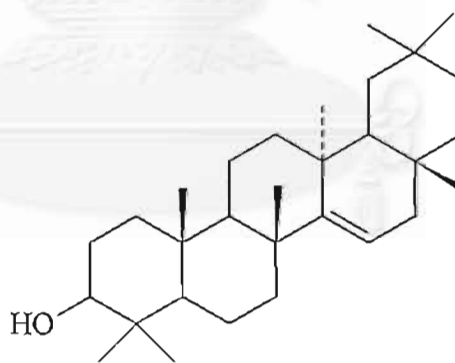
Structure of Compound 2

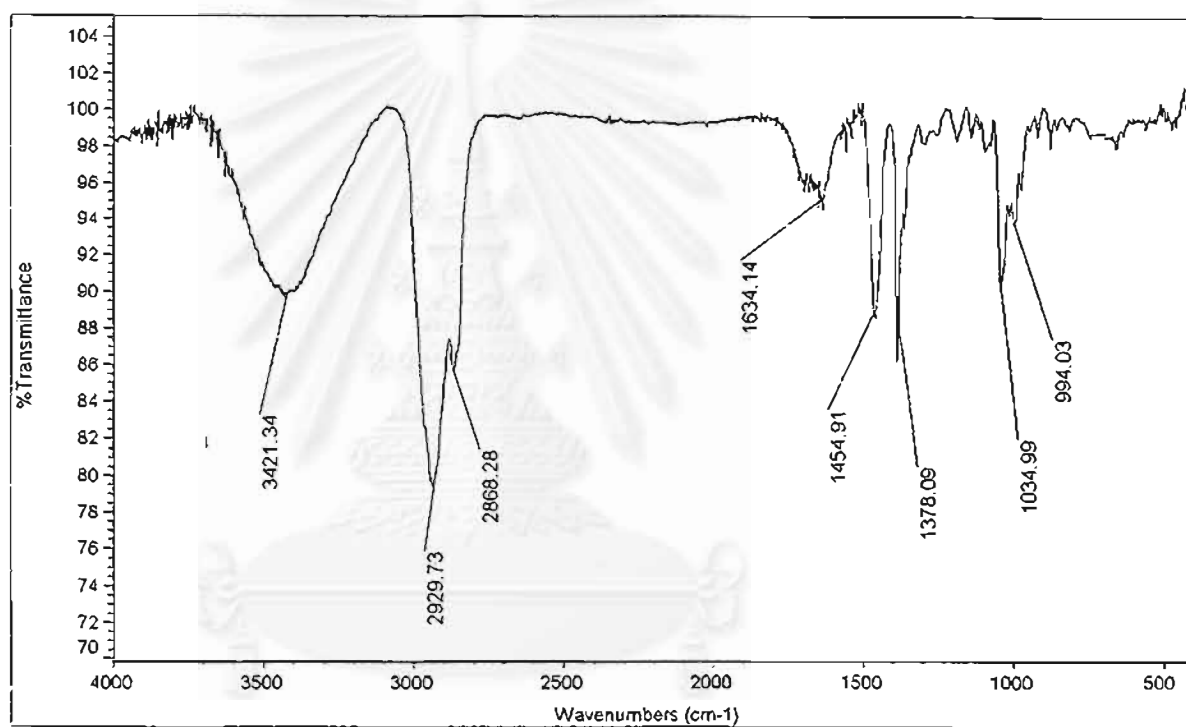**Scheme 3.2** Possible mass fragmentation of Compound **2**
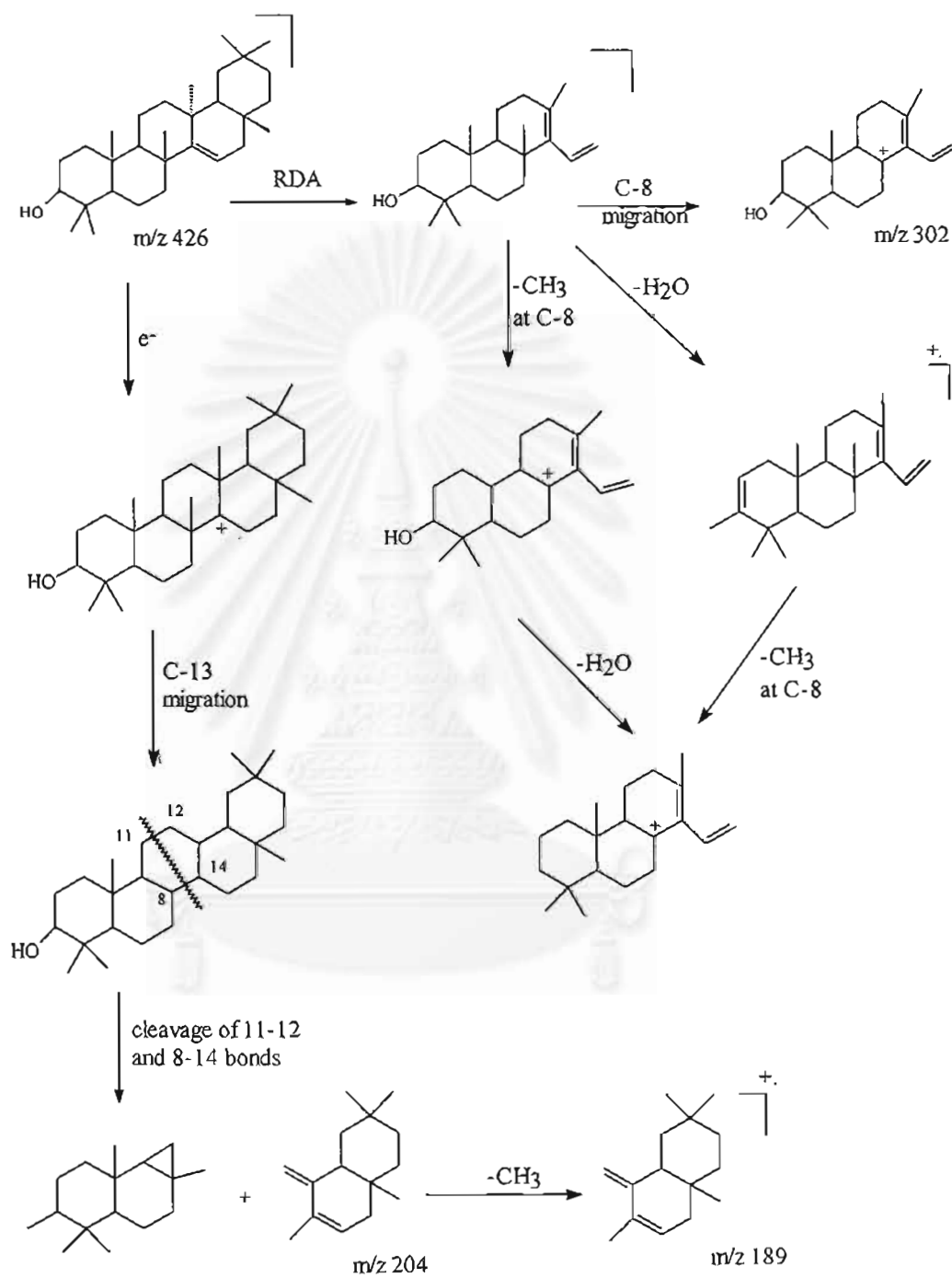
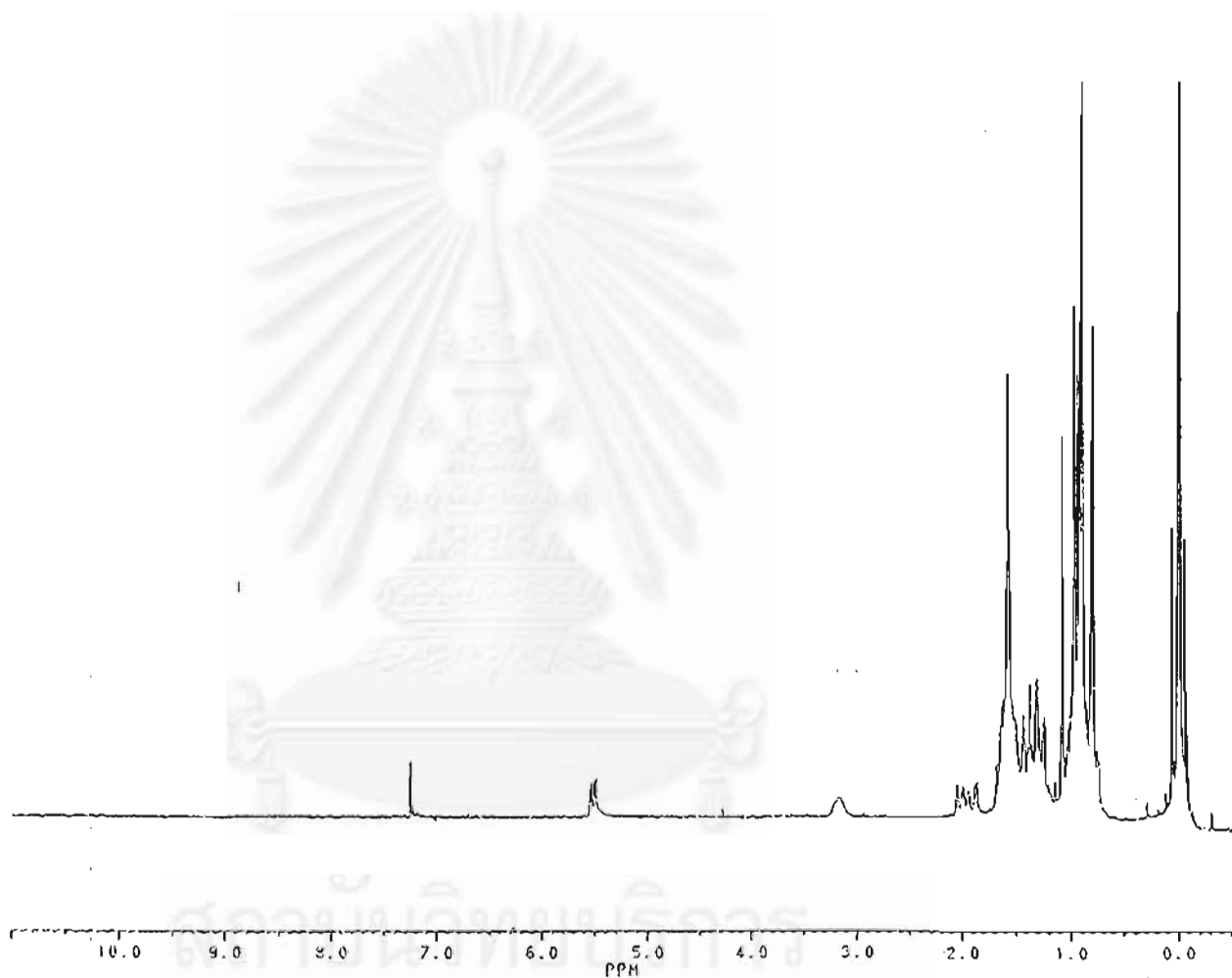**Fig. 3.5** The IR spectrum of Compound **2**

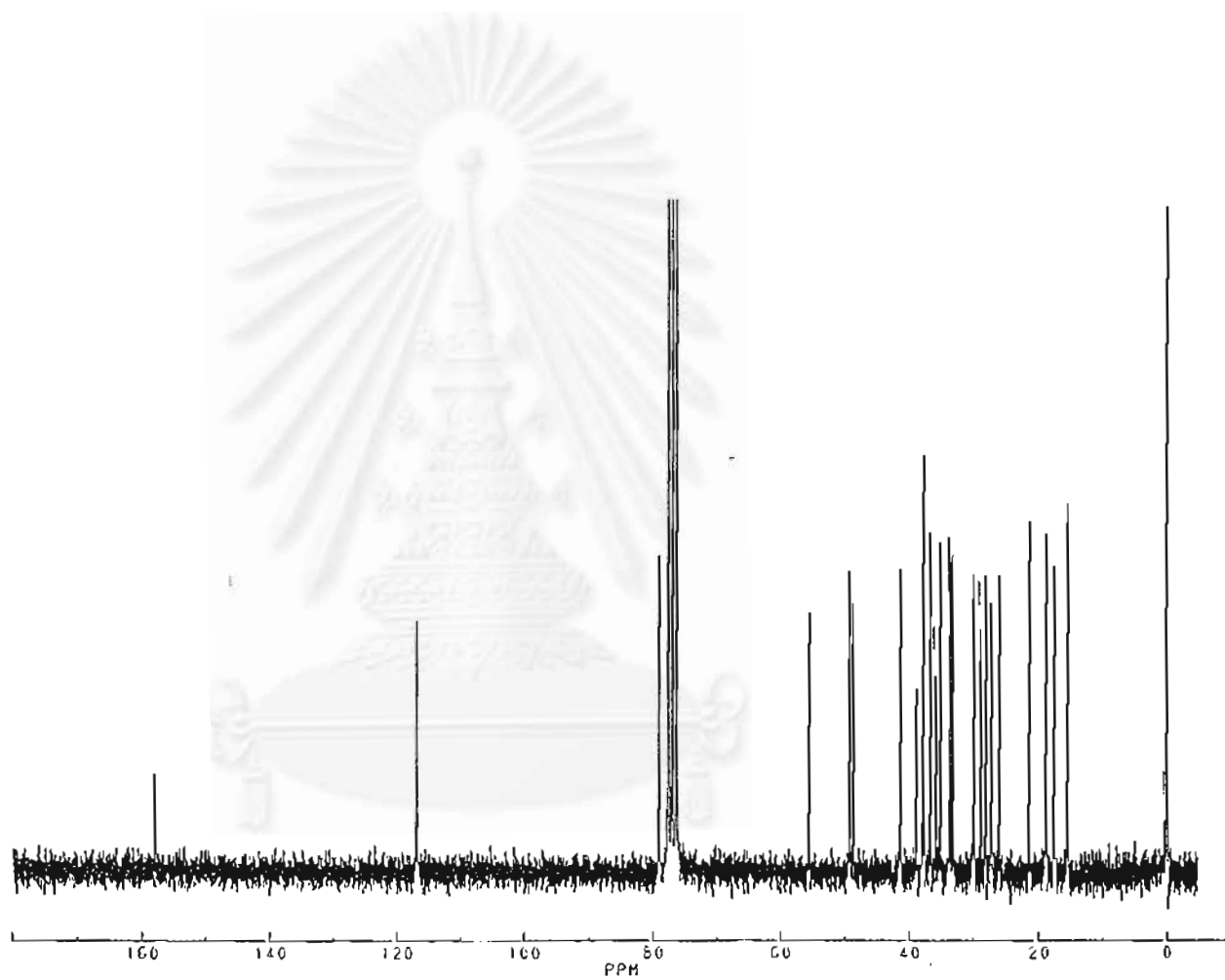Fig. 3.6 The ^{1}H NMR spectrum of Compound 2
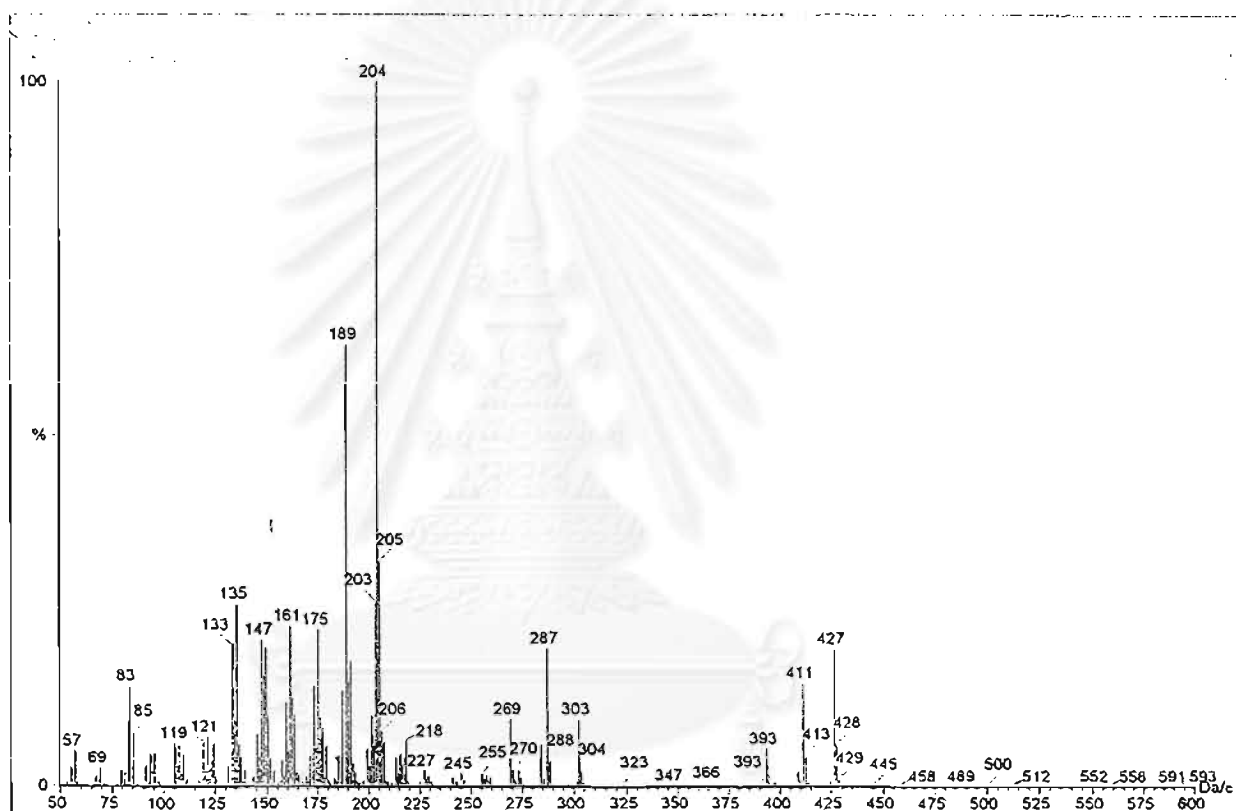
Fig. 3.7 The ^{13}C NMR spectrum of Compound 2

Fig. 3.8 The mass spectrum of Compound 2

3.4.3 Physical properties and structural elucidation of Compound 3

As a white solid powder, Compound **3** was gained after recrystallization from hot hexane (15 mg, 0.04% yield w/w) from Fr.No. 11 of the hexane crude extract of leaves. It was soluble in hexane, dichloromethane, and chloroform. It has an R_f value of 0.24 in $CHCl_3$ solvent system and is UV inactive. When chemically tested with Liebermann-Burchard's reagent, it gave a deep-pink result, illustrating the existence of triterpenoidal nucleus in this compound. Because of the small amount of this compound, further investigation of structure was not carried out.

3.4.4 Physical properties and structural elucidation of Compound 4

This compound was obtained from Fr.No. 2 and 3 of the hexane crude extract of the roots (26.7 mg, 0.07 % yield w/w) after column chromatography (10% EtOAc-Hex) and chromatotron separation (1% EtOAc-Hex). This compound was white-needle crystals. It has an R_f value of 0.66 in 10% EtOAc-Hex solvent system and was UV active when visualized by a UV lamp (254 nm). It was soluble in various solvents such as hexane, dichloromethane, chloroform and ethyl acetate. It also showed a positive result indicating the presence of triterpenoidal nucleus when tested with Liebermann-Burchard's reagent.

3.4.5 Physical properties and structural elucidation of Compound 5

Compound 5 was obtained from Fr.No.6 of the hexane crude extract of the roots (350 mg, 0.92 % yield w/w) and Fr.No 2 of the dichloromethane crude extract of the roots, (45 mg, 0.12% yield w/w). This compound consisted of needle-white crystal, m.p. 145-148 ^{0}C and had an R_f value 0.31 in 100% chloroform. It was soluble in dichloromethane and chloroform. When tested with Liebermann-Burchard's reagent, it gave a blue color, the positive result of steroid-containing compound.

The IR absorption bands (Fig.3.9) at 3600-3300 cm^{-1} suggested the presence of a hydroxyl group and at 969 cm^{-1} due to a disubstituted alkene. The ^{1}H NMR spectrum of Compound 5 showed signals for $-CH_3$, $-CH_2-$ and $-CH$ of steroid at δ 0.50-2.50, hydroxy group at δ 3.50, and the protons of $-CH=CH$ at δ 5.00 and 5.22 (Fig. 3.10). The ^{13}C NMR, DEPT 90 and 135 spectra indicated that this compound contained 6 methyl carbons, 8 methylene carbons, 11 tertiary carbons and 4 quarternary carbons (Fig. 3.11)

The chemical tests, IR spectrum, and NMR spectra led to the conclusion that Compound 5 could be a steroidal compound having a hydroxy group and a double bond. These results were consistent with those of stigmasterol. The ^{13}C NMR spectrum of Compound 5 was compared with that of stigmasterol to authenticate the structure (Table 3.16).

Table 3.16 ^{13}C NMR chemical shifts of stigmasterol [21] compared to those of Compound 5

carbon No.	stigmasterol	Compound 5
1	37.4	37.3
2	31.7	31.7
3	71.8	71.8
4	42.4	42.3
5	140.0	140.7
6	121.7	121.7
7	31.9	31.9
8	31.9	31.9
9	50.3	50.1
10	36.6	36.5
11	21.1	21.2
12	39.8	39.8
13	42.4	42.3
14	57.0	56.9
15	24.4	24.3
16	28.9	28.9
17	56.0	55.9
18	12.2	12.2
19	19.4	19.4
20	40.5	40.5
21	21.1	21.2
22	138.4	138.3
23	129.4	129.3
24	51.3	51.2
25	31.9	31.9
26	19.0	19.1
27	21.1	21.1
28	25.4	25.4
29	12.0	12.0

It can be concluded that this compound is stigmasterol, and its structure is shown below.
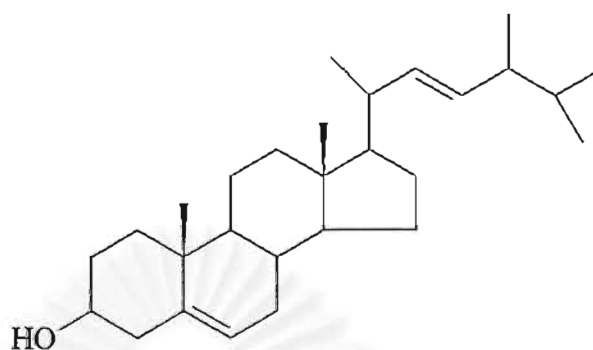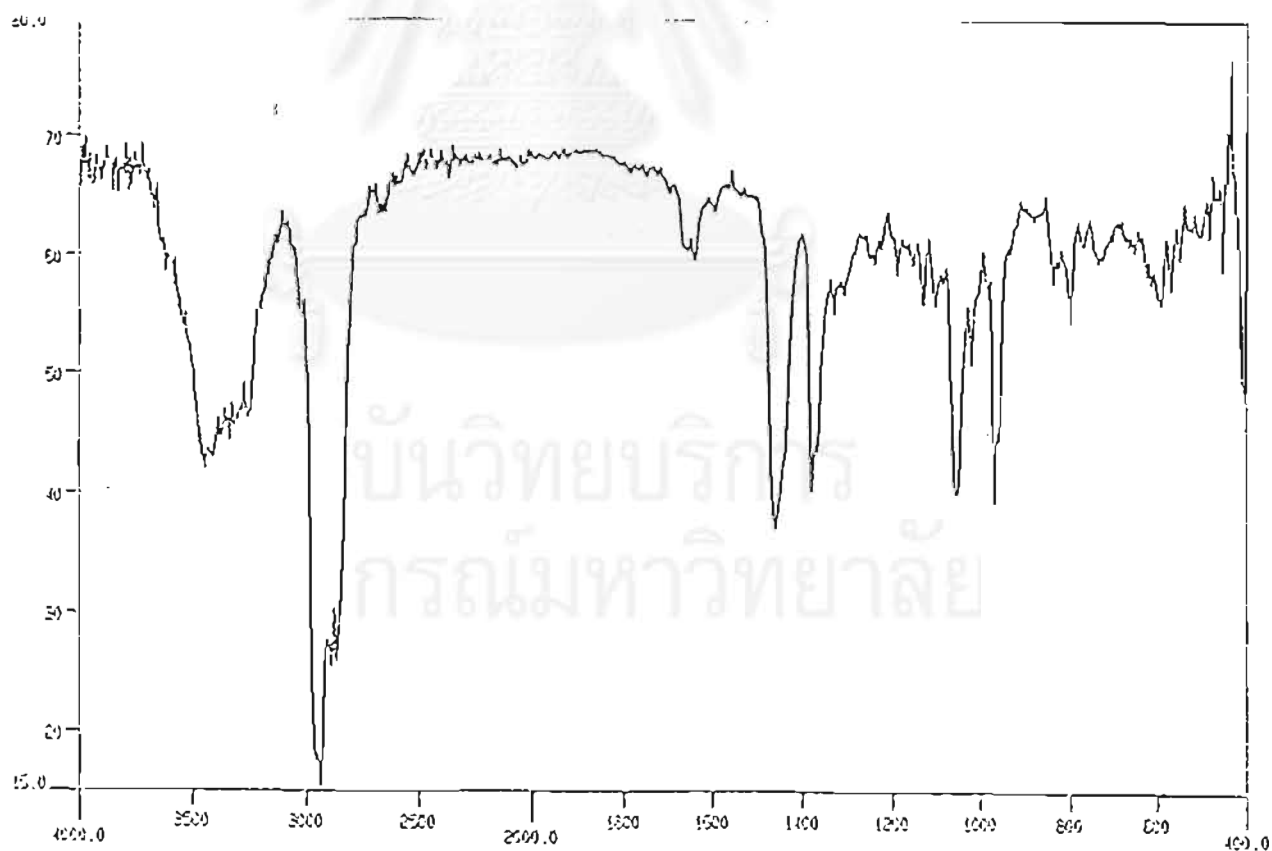

Structure of Compound 5

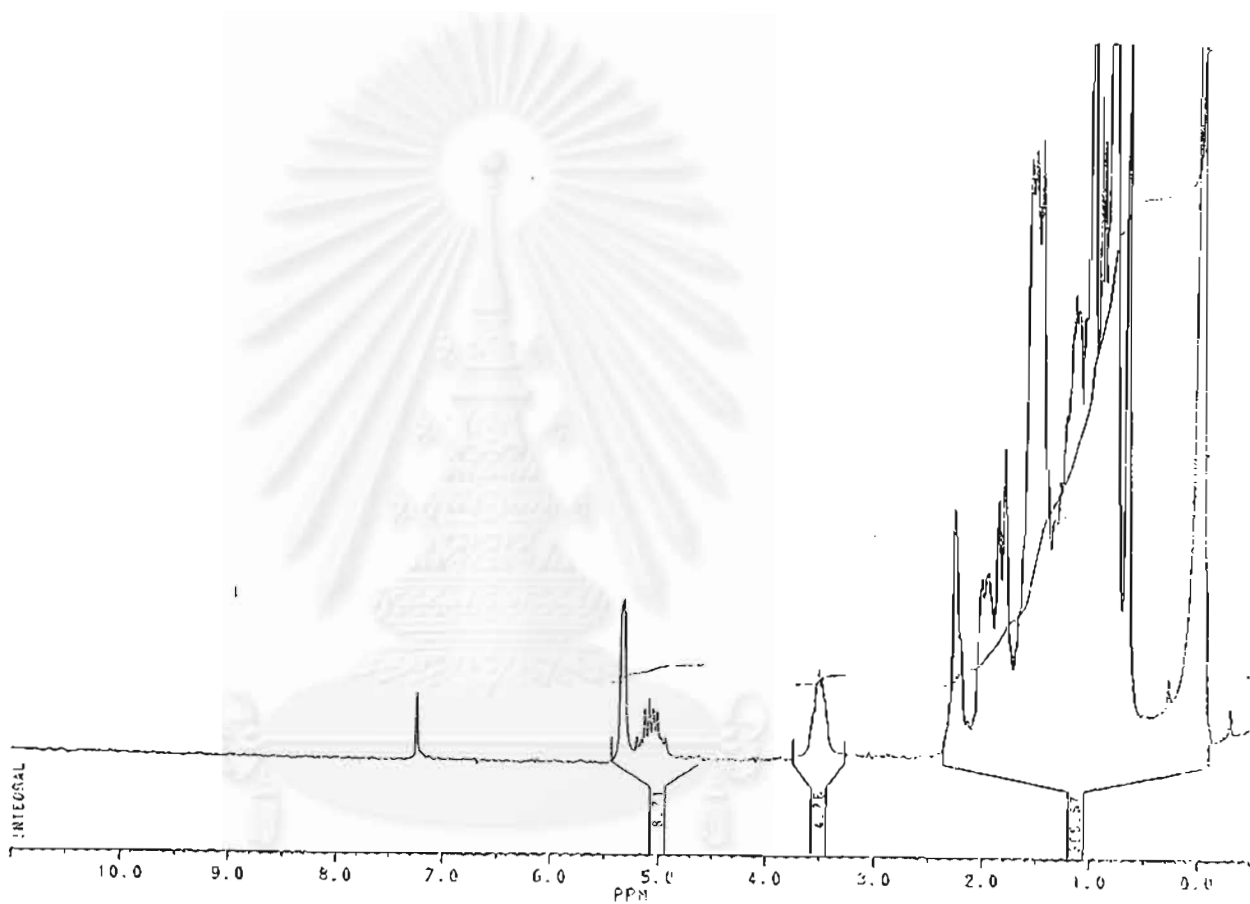**Fig. 3.9** The IR spectrum of Compound 5

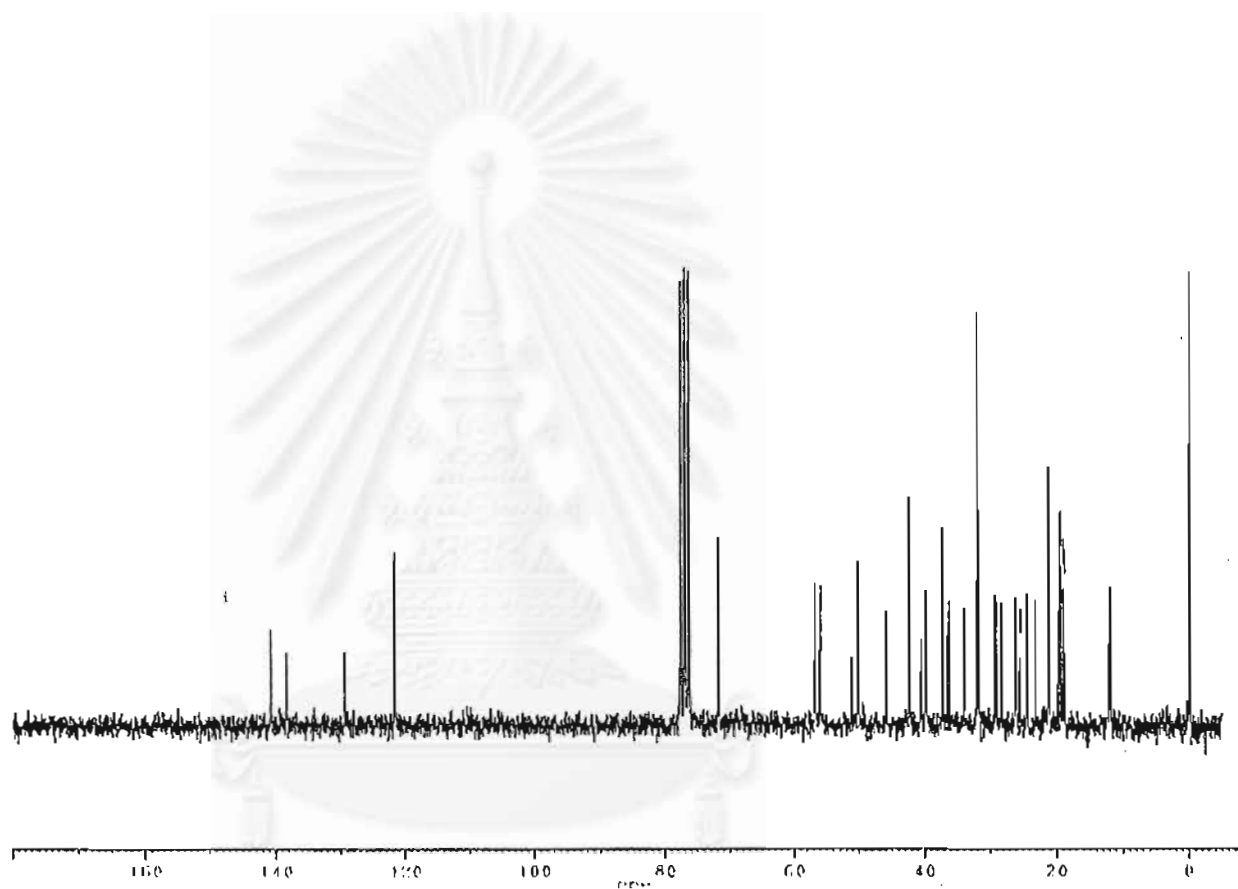**Fig. 3.10** The ^{1}H NMR spectrum of Compound **5**

Fig. 3.11 The ^{13}C NMR spectrum of Compound 5

3.4.6 Physical properties and structural elucidation of Compound 6

Compound 6 was separated from Fr.No. 5, (60 mg, 0.15% yield w/w of dried roots). It yielded the positive results with Maeyer's, Wagner's, Dragendorff's ,and Marme's reagents. This compound was recrystallized from 70:30 MeOH: $CHCl_3$ to give colorless-needle solid, m.p. 200-202 $^{\circ}C$, with R_f value of 0.42 in 50% EtOAc/$CHCl_3$ solvent system. From the result of chemical reactions, Compound 6 could be an alkaloid.

The broad IR absorption (Fig. 3.13) band at 3600-3300 cm^{-1} suggested the presence of amine group in the molecule and that at 1673 cm^{-1} suggested the presence of carbonyl group. Mass spectrometry (Fig. 3.17) indicated m/z= 144 $(M-1)^+$ which could be an alkaloid with one nitrogen atom. The mass spectrum showed fragments at 144, 116, 89, 50, and 39 which m/z 116 $(C_8H_6N)^+$ is the characteristic signal of indole nucleus.

The ^{1}H-NMR spectrum(Fig. 3.14) displayed signals at δ 7.26 (2H, m), δ 7.54 (1H, d, J = 7.30 Hz), δ 8.19 (1H, s), δ 8.23 (1H, d, J = 7.02 Hz), and δ 10.03 (1H,s). The last signal was corresponded to an aldehyde proton. The ^{13}C NMR, DEPT 90 and DEPT 135 spectra (Fig. 3.15 and 3.16) displayed signals of 9 carbons, all of which are sp^2 carbons as follows: one carbonyl carbon at δ 185.3, five methine carbons at δ 112.9, 122.2, 123.0, 124.5, and 137.9 and three quartemary carbons at δ 120.1, 125.5 and 138.2. According to the data above, it can be deduced that this compound would be indole alkaloid bearing one aldehyde group. The aldehyde group containing in the molecule could be in either α- or β- positions.

α- derivative (m.p. 140-142 °C)   β- derivative (m.p. 195-198 °C)

**Fig. 3.12** The two possible structures of 1H-indole-carboxaldehyde

The melting point of α-derivative was 140-142 °C,[22] while that of β-derivative was 195-198 °C [23] which is closer to this compound. Furthermore, a library search data of mass spectrometry and comparison of ^{13}C-NMR chemical shifts of both Compound **6** and 1-H-indole-carboxaldehyde (Table 3.17) confirmed this compound to be indole alkaloid compound (C_9H_7NO, 1H-indole-3-carboxaldehyde)

Table 3.17 The ^{13}C NMR chemical shifts of 1H-indole-3-carboxaldehyde[24] and those of Compound **6**

carbon No.	1H-Indole-3-carboxaldehyde[A]	Compound 6[B]
C-2	139.1	137.9
C-3	118.8	120.1
C-4	124.5	124.5
C-5	122.9	122.9
C-6	121.5	122.1
C-7	113.1	112.9
C-3a	124.7	125.5
C-7a	137.7	138.2
C-3-CHO	186.0	185.3

A- d_6- DMSO as a solvent   B- d_6-acetone as a solvent

This compound, therefore, should be 1H-indole-3-carboxaldehyde. The structure is shown below.
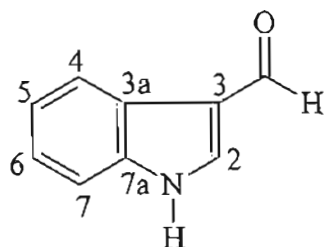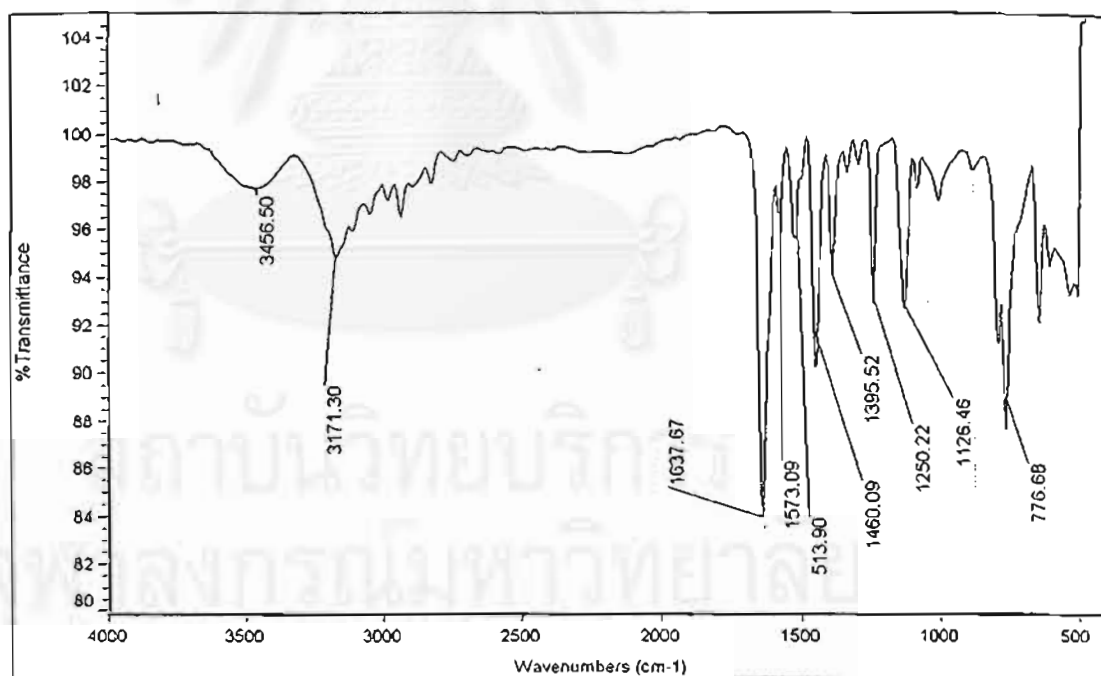
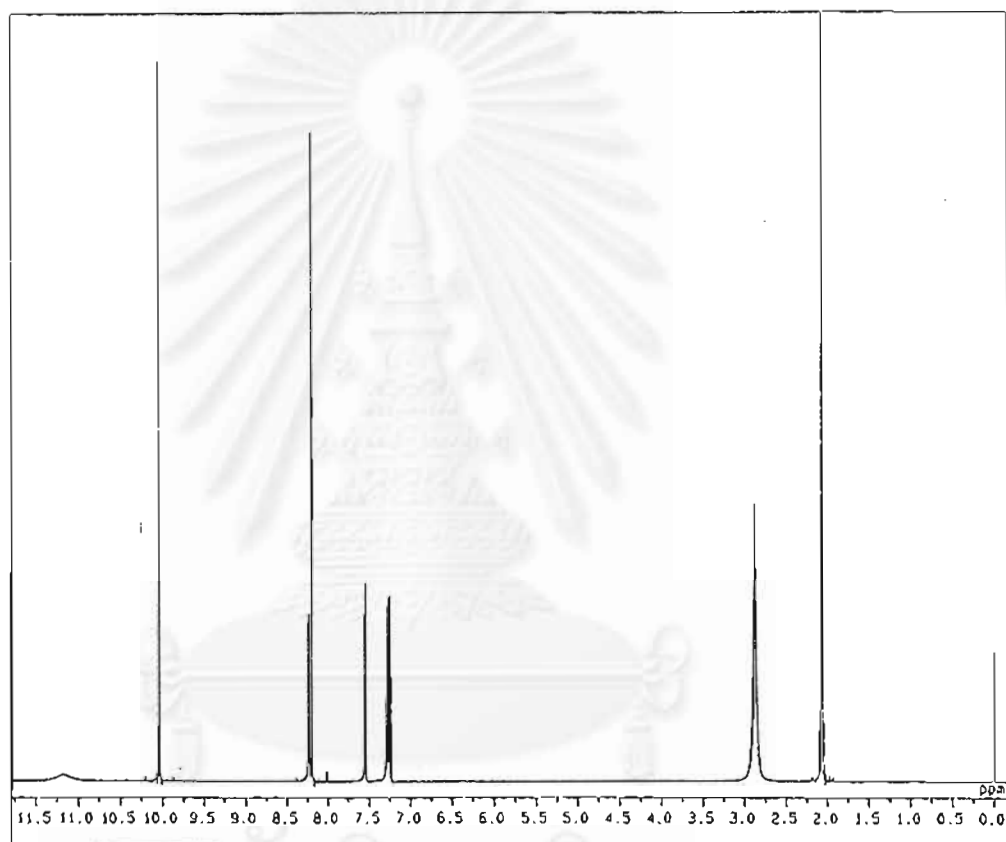**The structure of Compound 6**

Fig. 3.13 The IR spectrum of Compound 6
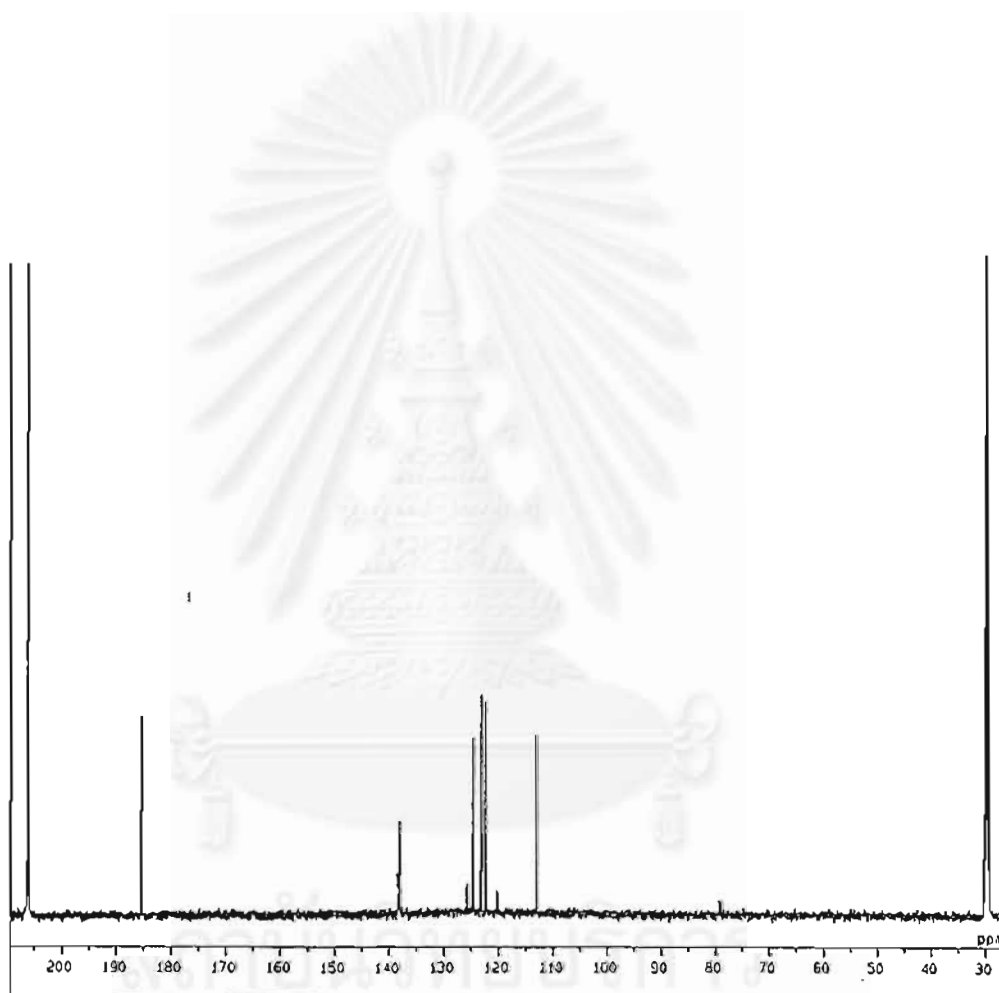
Fig. 3.14 The ^{1}H NMR spectrum of Compound 6

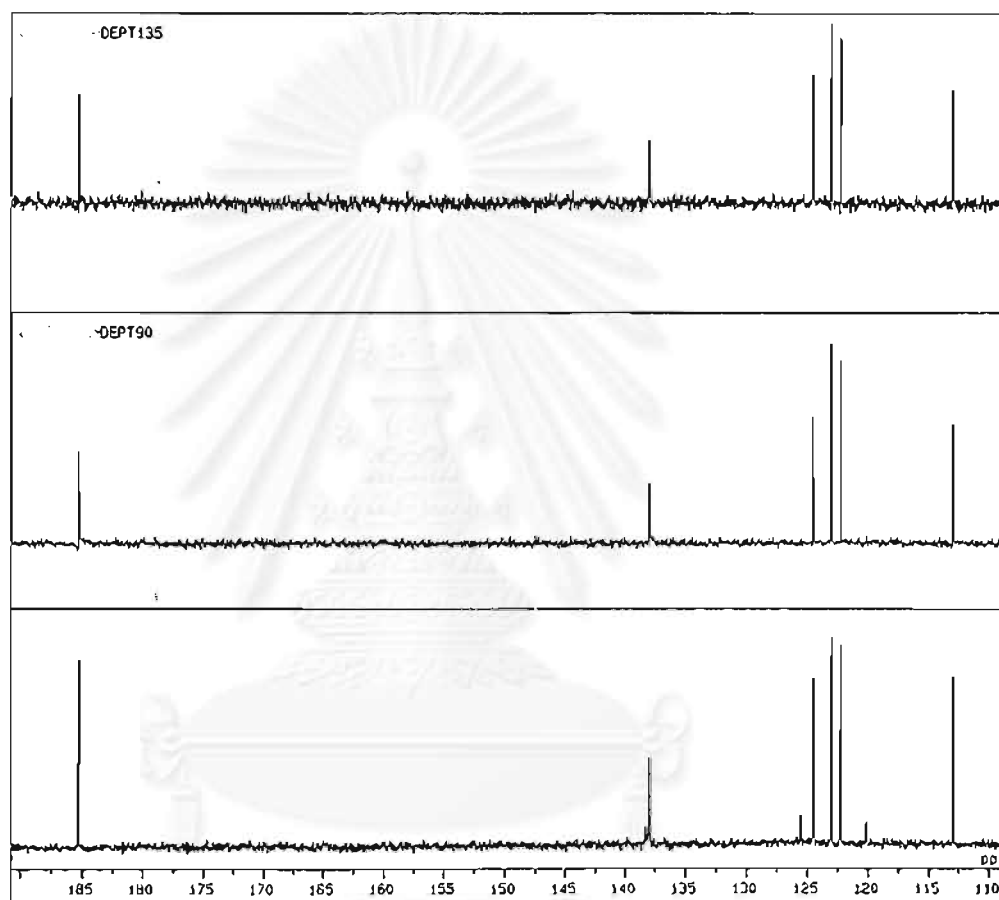**Fig. 3.15** The ^{13}C NMR spectrum of Compound **6**

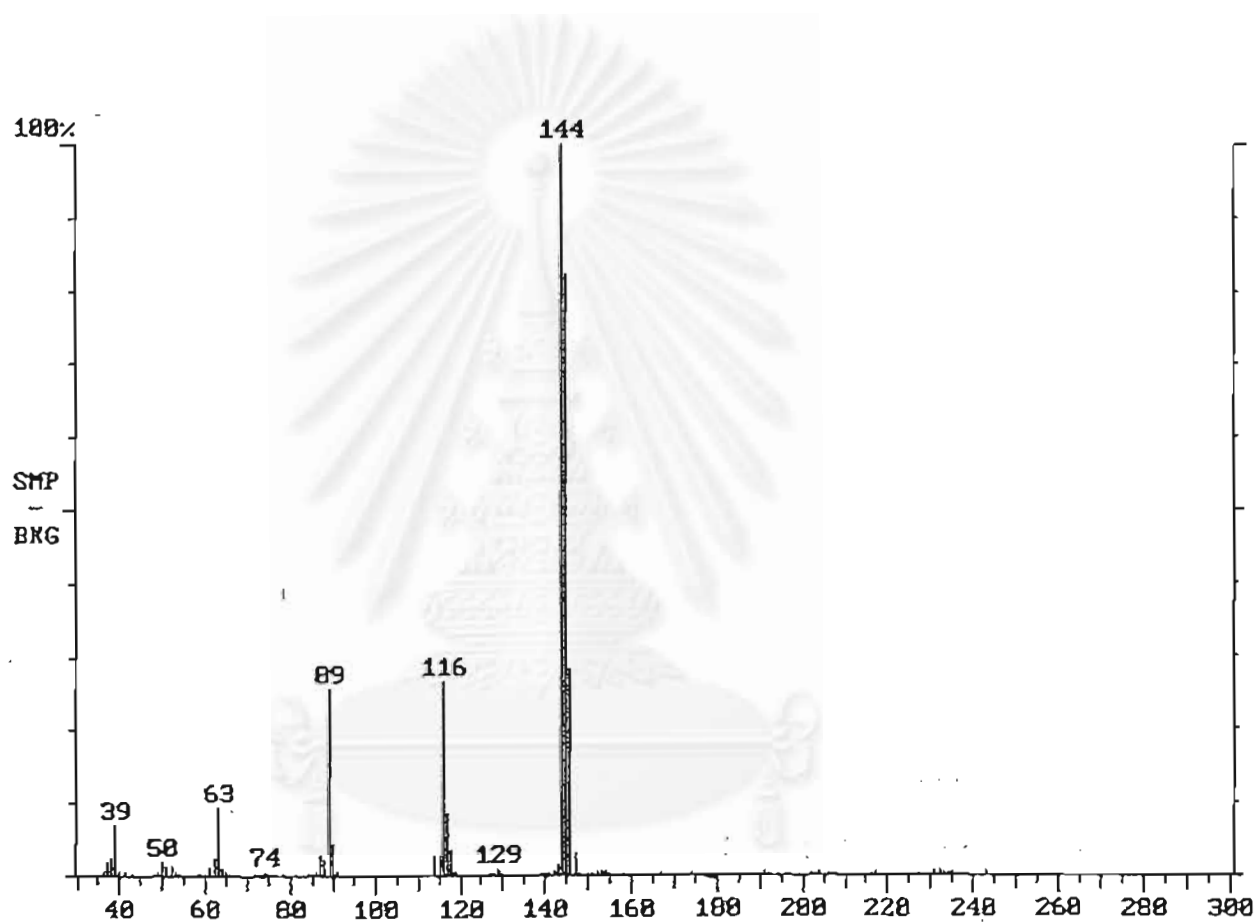Fig. 3.16 The DEPT 90 and 135 spectra of Compound 6

Fig. 3.17 The mass spectrum of Compound **6**

3.4.7 Physical properties and structural elucidation of Compound 7

Compound 7 is orange liquid (20 mg, 0.05% yield w/w of the hexane crude extract of the roots) and isolated from the hexane crude extract of roots. It was soluble in CH_2Cl_2, EtOAc, and had an R_f value of 0.58 in 20% EtOAc/CHCl$_3$ solvent system. It gave a positive result to Maeyer's, Wagner's, Dragendorff's, and Marme's reagents.

In the ^1H NMR spectrum (Fig. 3.19), there were signals at δ 4.19 (OCH$_3$,s), δ 7.33 (1H, dt, J = 1.2, 7.3 Hz), δ 7.38 (1H, dt, J = 1.2, 7.0 Hz), δ 7.48 (1H, d, J = 8.24 Hz), δ 7.89 (1H, s), δ 8.31 (1H, d, J = 7.6 Hz) and δ 9.96 (1H,s). The ^{13}C NMR, DEPT 90 and DEPT 135 (Fig. 3.20 and 3.21) displayed one methoxy carbon at δ 66.8, five olefinic methine carbons at δ 131.7, 124.6, 123.5, 122.1 and 108.7, three olefinic quarternary carbons at δ 132.6, 121.6 and 114.0 and one aldehyde carbon at δ 184.1. It could be established that the molecular formula of this compound was $C_{10}H_9NO$ from the results of elemental analysis (anal. C 68.55%, H 5.21%, N 7.91%, calcd. for $C_{10}H_9NO_2$ C 68.57%, H 5.14%, N 8.00%)

The comparison of the ^1H and ^{13}C NMR spectra of Compounds 6 and 7 showed the same patterns, except for the presence of the additional methoxy group in Compound 7. The molecular ion of m/z 175 is 30 mass units higher than the corresponding molecular peak of Compound 6, which supported the substitution of methoxy group including onto Compound 6. This substituent could be placed at any position on the indole skeleton. When considering the ^1H NMR pattern, the spectrum had the signals of an o-disubstituted benzene ring.[25] This methoxy group, therefore, cannot substitute on any position of the benzene moiety.
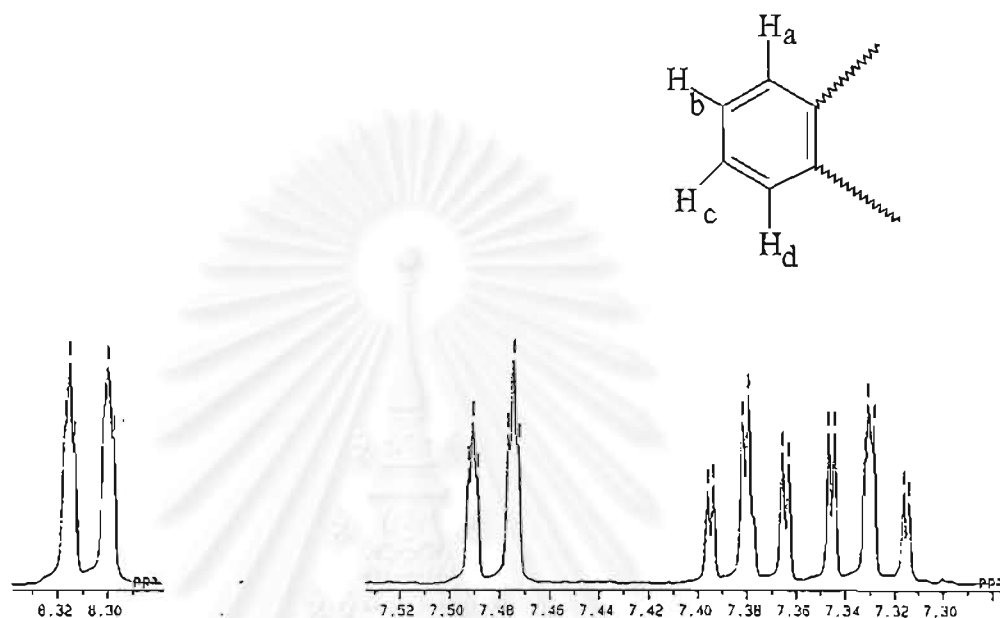
Fig. 3.18 Illustration of proton signals of *o*-disubstituted benzene ring in Compound 7

In general, the ^{13}C chemical shift of any C-methoxy group could be around 55-57 ppm, but this methoxy group resonated at δ 66.8 ppm, which is unusually down field. This phenomenon can be clarified by a literature survey that the methoxy group might connect to the N atom of the indole structure.[26] The unusual N-methoxyindole structure has been observed in the tryptamine alkaloid from *Lespedeza bicolor* var. japonica (family Leguminasae) and in neoglucobrassicin from *Brassica napus* L. var. napobrassica.[27]

It can be concluded that Compound 7 should be 1-methoxy-indole-3-carboxaldehyde. Its structure is shown below.
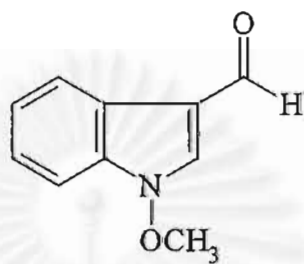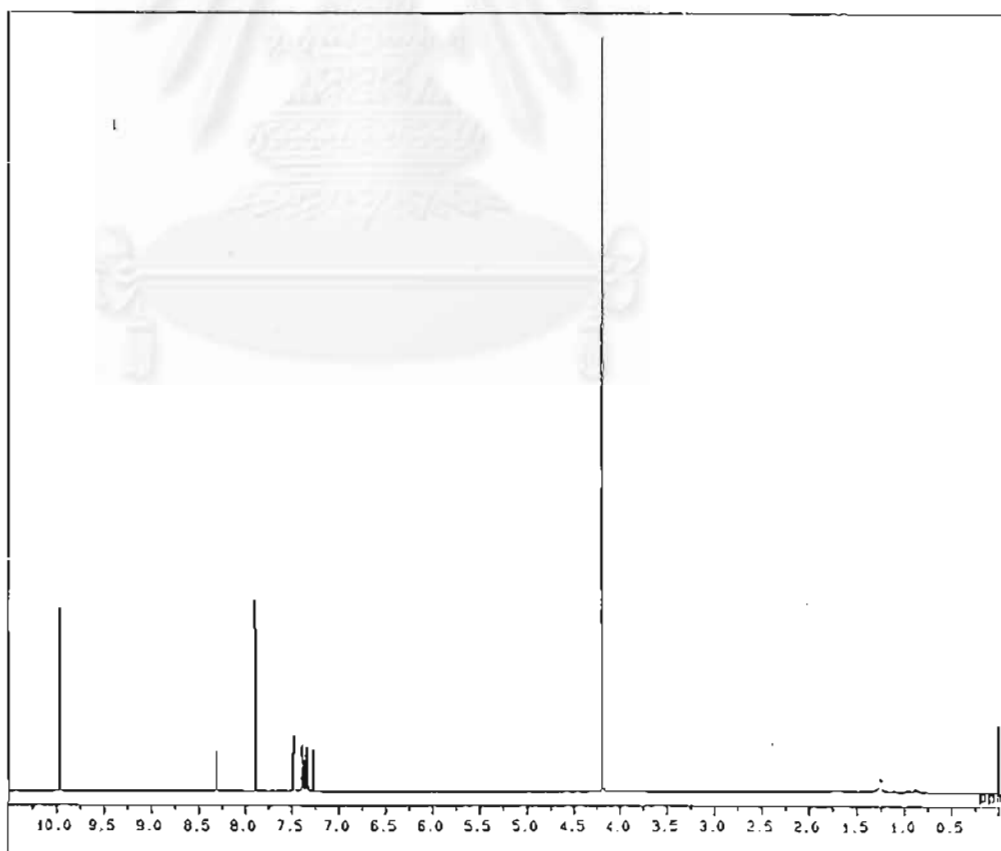

The structure of Compound 7

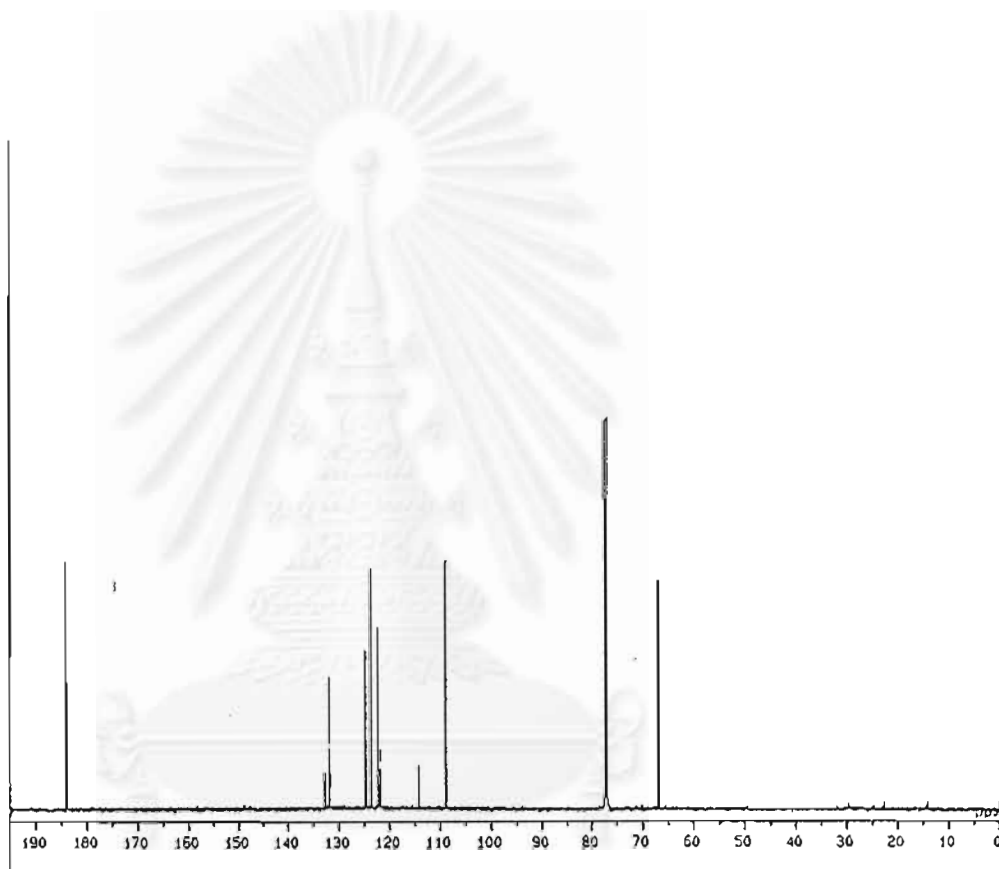**Fig. 3.19** The ^{1}H NMR spectrum of Compound 7

Fig. 3.20 The ^{13}C NMR spectrum of Compound **7**
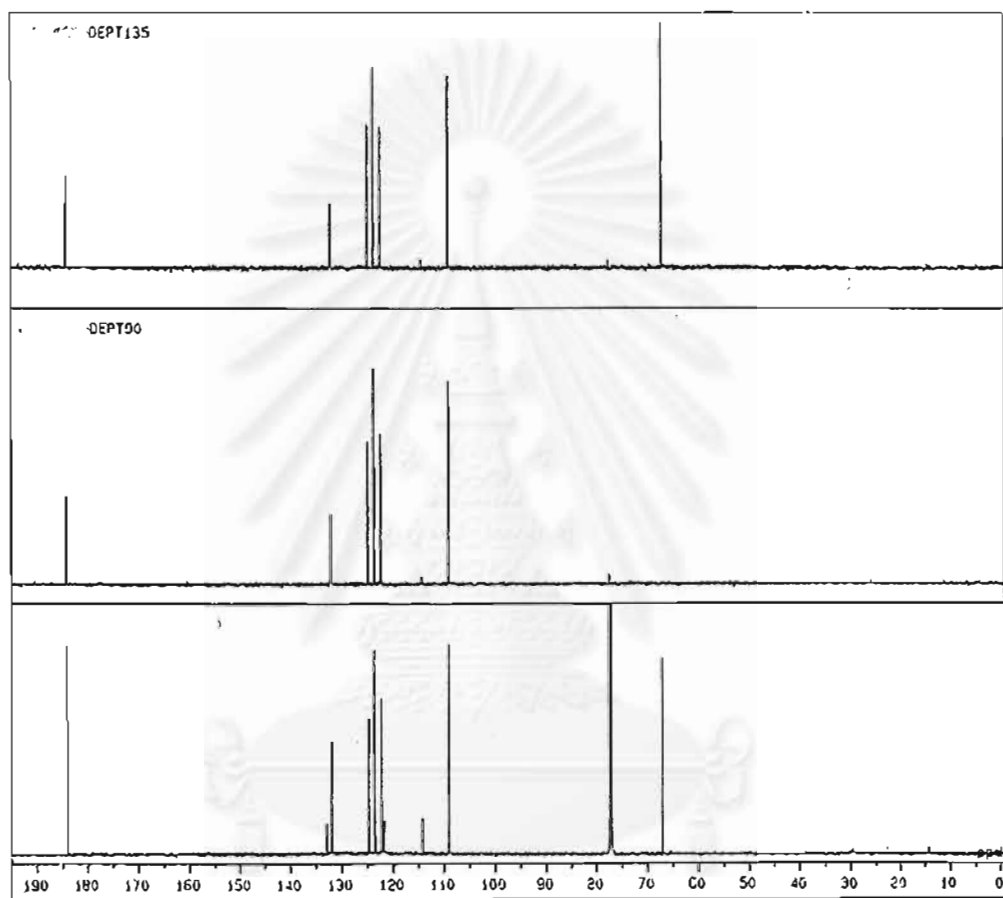
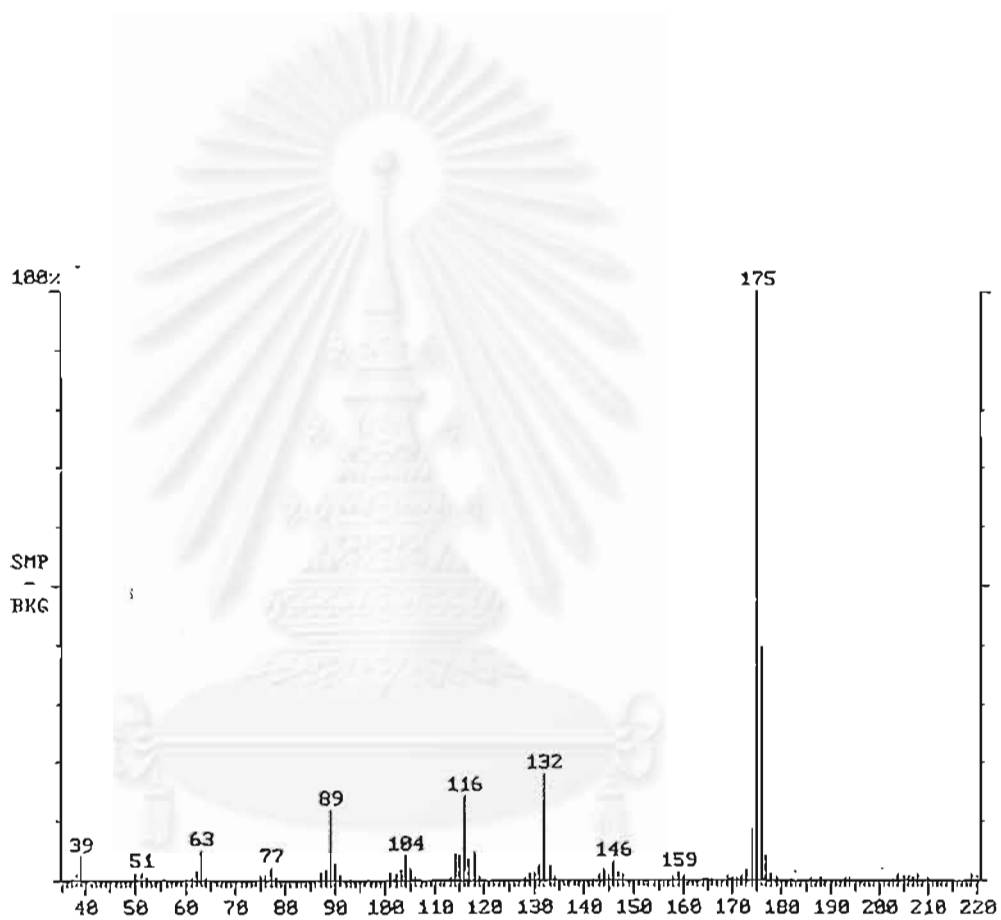**Fig. 3.21** The DEPT 90 and 135 spectra of Compound 7

Fig. 3.22 The mass spectrum of Compound 7

3.4.8 Physical properties and structural elucidation of Compound 8

As an orange liquid, Compound 8 was obtained from Fr.No. 1, 2 and 3 of the dichloromethane crude extract of the roots (80 mg, 0.21 % yield w/w). It was soluble in CH_2Cl_2, $CHCl_3$ and EtOAc and had an R_f value of 0.66 in 70% $CHCl_3$-Hex solvent system. It showed the positive results to Maeyer's, Wagner's, Dragendorff's, and Marme's reagents.

The ^1H NMR spectrum (Fig. 3.27) and signal integration indicated the presence of methylene protons at δ 3.79 (2H, d, J= 1.2 Hz), methoxy protons at δ 4.08 (3H, s) including with olefinic protons at δ 7.17 (1H, dt, J= 0.9-1.8, 7.3-7.9 Hz), δ 7.30 (1H, s, J= 1.2 Hz), δ 7.30 (1H, dd, J= 0.9-1.2, 15.0 Hz), δ 7.45 (1H, td, J= 0.9, 8.2 Hz), and δ 7.55 (1H, td, J= 0.9, 7.9 Hz)

The ^{13}C NMR, DEPT 90 and 135 spectra of this compound (Fig. 3.30 and 3.31) showed carbon signals as follows: a methylene carbon at δ 14.2 ppm, a methoxy carbon at δ 66.0 ppm, five olefinic methine carbons at δ 108.6, 118.3, 120.4, 121.7, and 123.2 and four olefinic quarternary carbons at δ 100.3, 117.8, 122.4 and 132.4 ppm.

Thus, this compound has 11 carbon atoms, 10 hydrogen atoms and one oxygen atom ($C_{11}H_{10}O$ = 158), which does not match with the molecular ion peak (m/z = 186). From elemental analysis, it can be deduced that in fact the molecular formula is $C_{11}H_{10}N_2O$ (anal. C 71.02%, H 5.53%, N 14.94%, calcd. for $C_{11}H_{10}N_2O$, C 70.97%, H 5.38%, N 15.05%) which does correspond to the observed molecular ion peak in the mass spectrum (fig. 3.28) ($C_{11}H_{10}N_2O$, m/z = 186). However, there are two ion peaks at m/z 171 [M-CH₃] and 155 [M-OMe]⁺ which might be the consequence of the lose of a methoxy group from the molecular ion. Due to the unusually down field shift of the methoxy group, it was suspected again that this methoxy group was connected to N atom.[26]
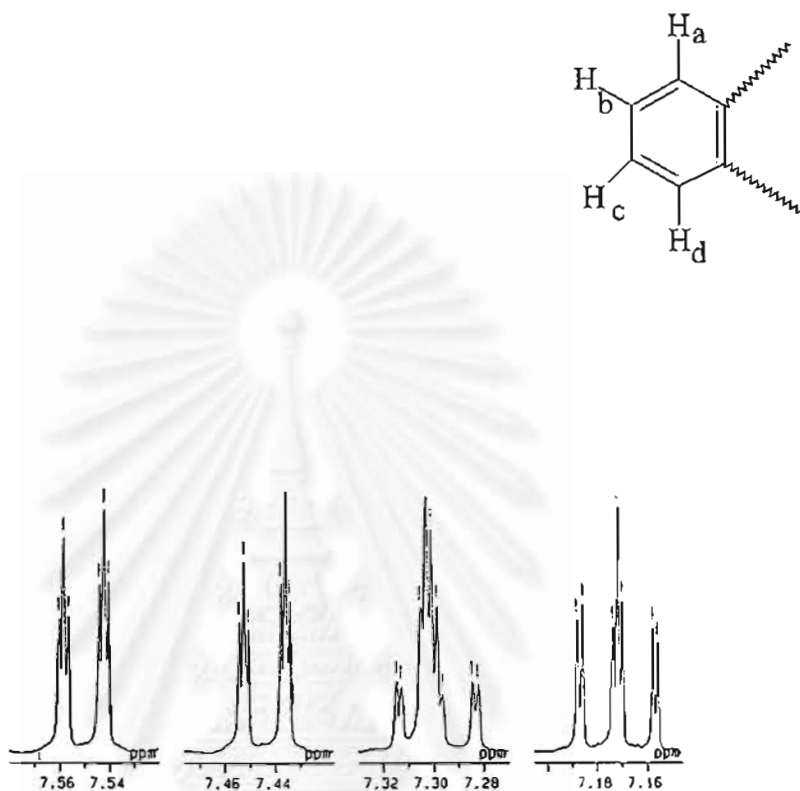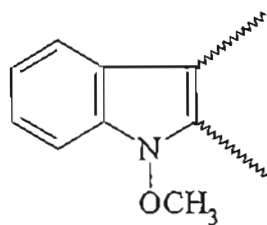
Fig.3.23 Illustration of proton signals of *o*-disubstituted benzene ring in Compound **8**

Like Compounds **6** and **7**, there was a group of proton signal, characteristic of an *o*-disubstituted benzene ring in Compound **8**. Therefore, it can be concluded that there was an indole moiety in molecule of Compound **8**.

From the IR spectrum (Fig. 3.29), there was the absorption band at 2248 cm^{-1}, which is the characteristic absorption band of a nitrile group. Therefore, there are two parts, methylene group and nitrile which have not been established in the moleclue. The two possible structures of Compound **8** are shown below.
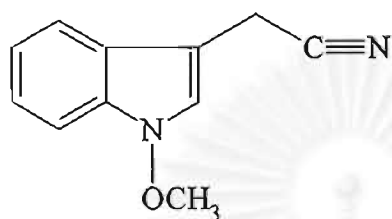
structure a
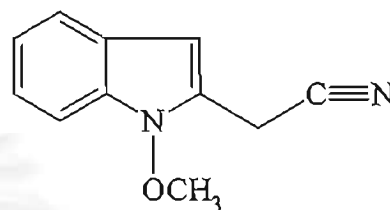
structure b

The NOESY spectrum (Fig. 3.24) indicated that structure **a** was the most likely because it showed the most possible correlation of protons (Fig. 3.24). It can be concluded that Compound **8** is 1-methoxy-indole-3- acetonitrile. The structure is shown below.
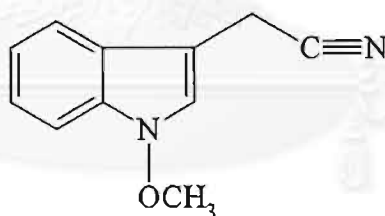
structure a

Structure of Compound 8
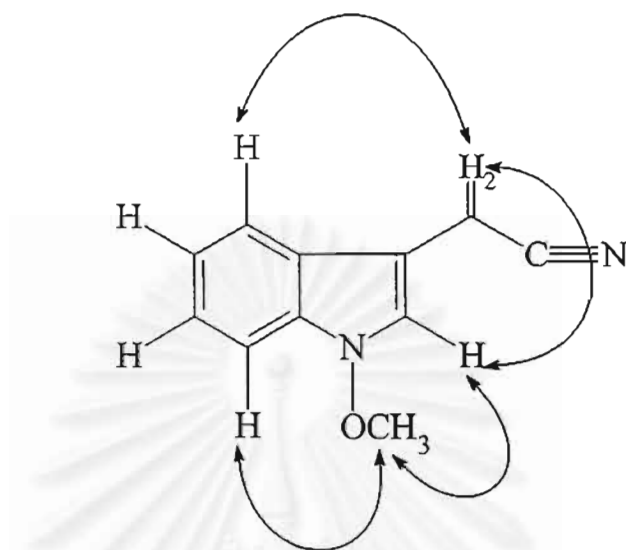
Fig.3.24 Through space coupling of protons as deduced from NOESY spectrum

The proton and carbon assignment can be deduced from the information of HMBC, HMQC, NOESY, ^{1}H-^{1}H COSY and shown below.
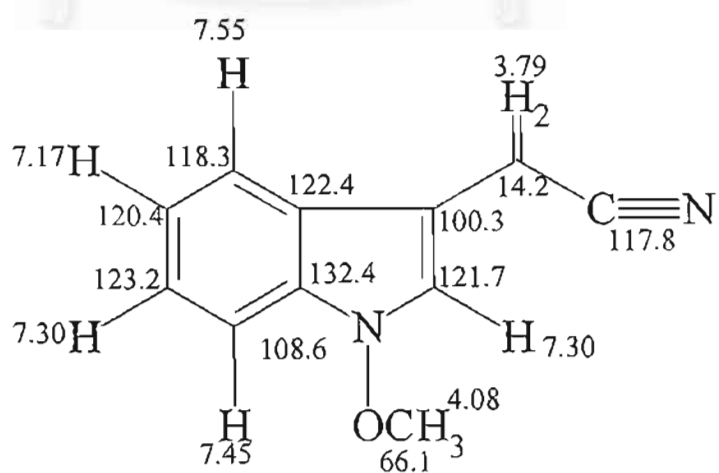
Fig.3.25 Proton and carbon assignment of Compound **8**

Table 3.18 The carbon and attached proton determined by one bond correlation in HMQC spectrum

Chemical shift	
Carbon	**Attached proton**
132.4	-
123.2	7.30(1H, dd, J = 0.9-1.2, 15.0 Hz)
122.4	-
121.7	7.30(1H, q, J = 1.2 Hz)
120.4	7.17(1H, dt, J = 0.9-1.8, 7.3-7.9 Hz)
118.3	7.55(1H, td, J = 0.9, 7.9 Hz)
117.8	-
108.6	7.45(1H, td, J = 0.9-8.2)
100.3	-
66.0	4.08(3H, s)
14.2	3.79(2H,d, J = 1.2 Hz)
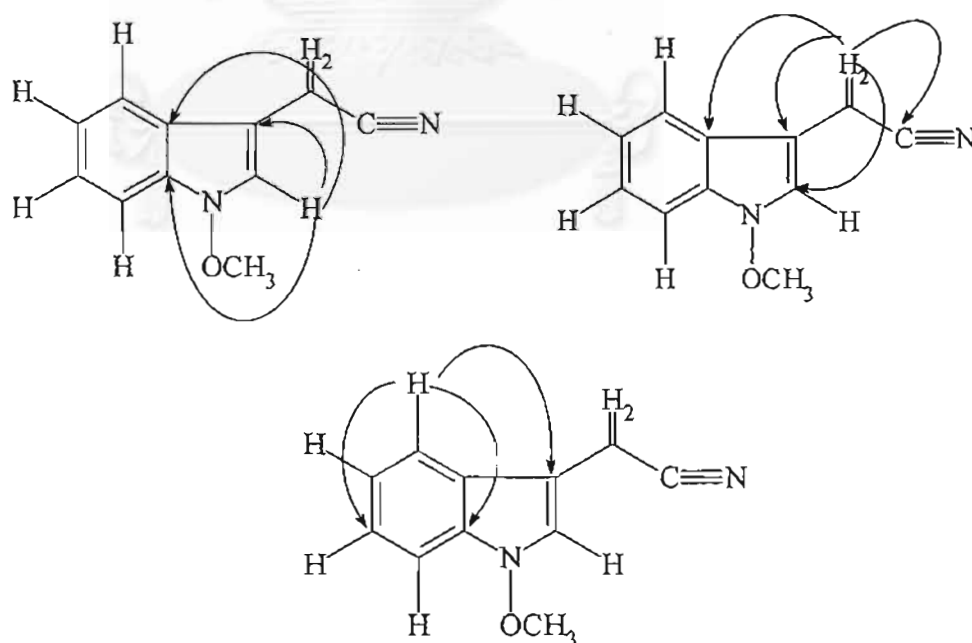
Fig.3.26 Long ranged ^{1}H-^{13}C coupling as detected in HMBC spectrum
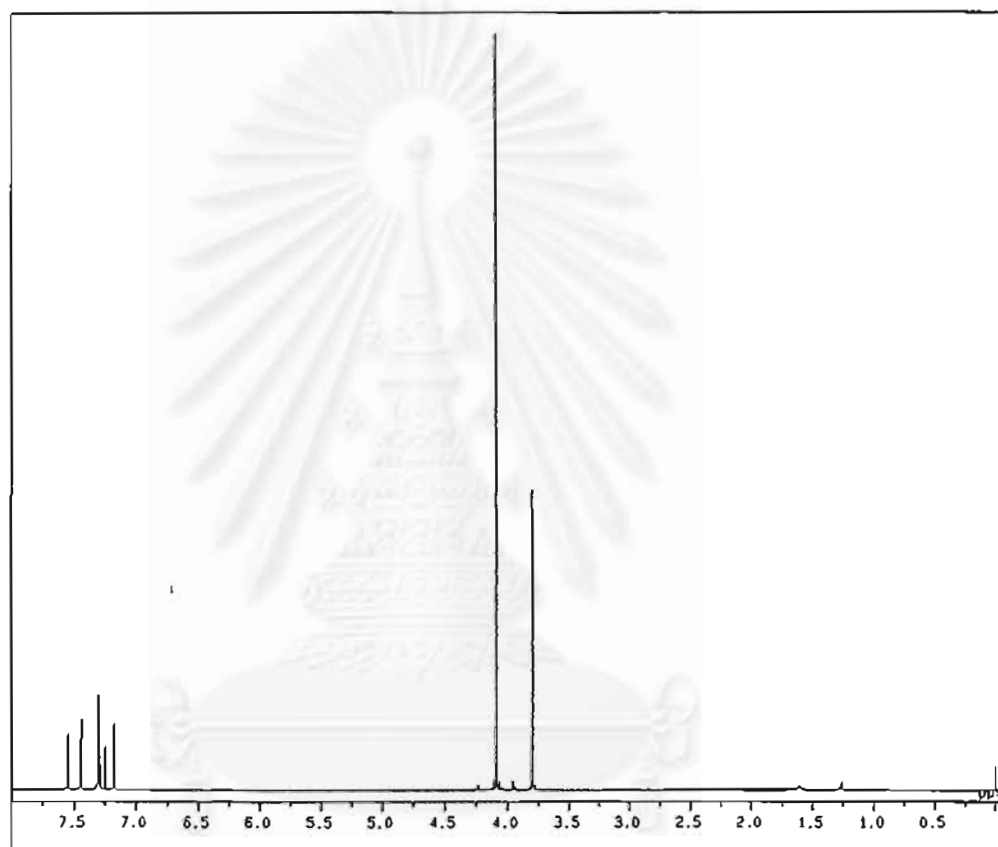
Fig.3.27 The ^{1}H NMR spectrum of Compound 8
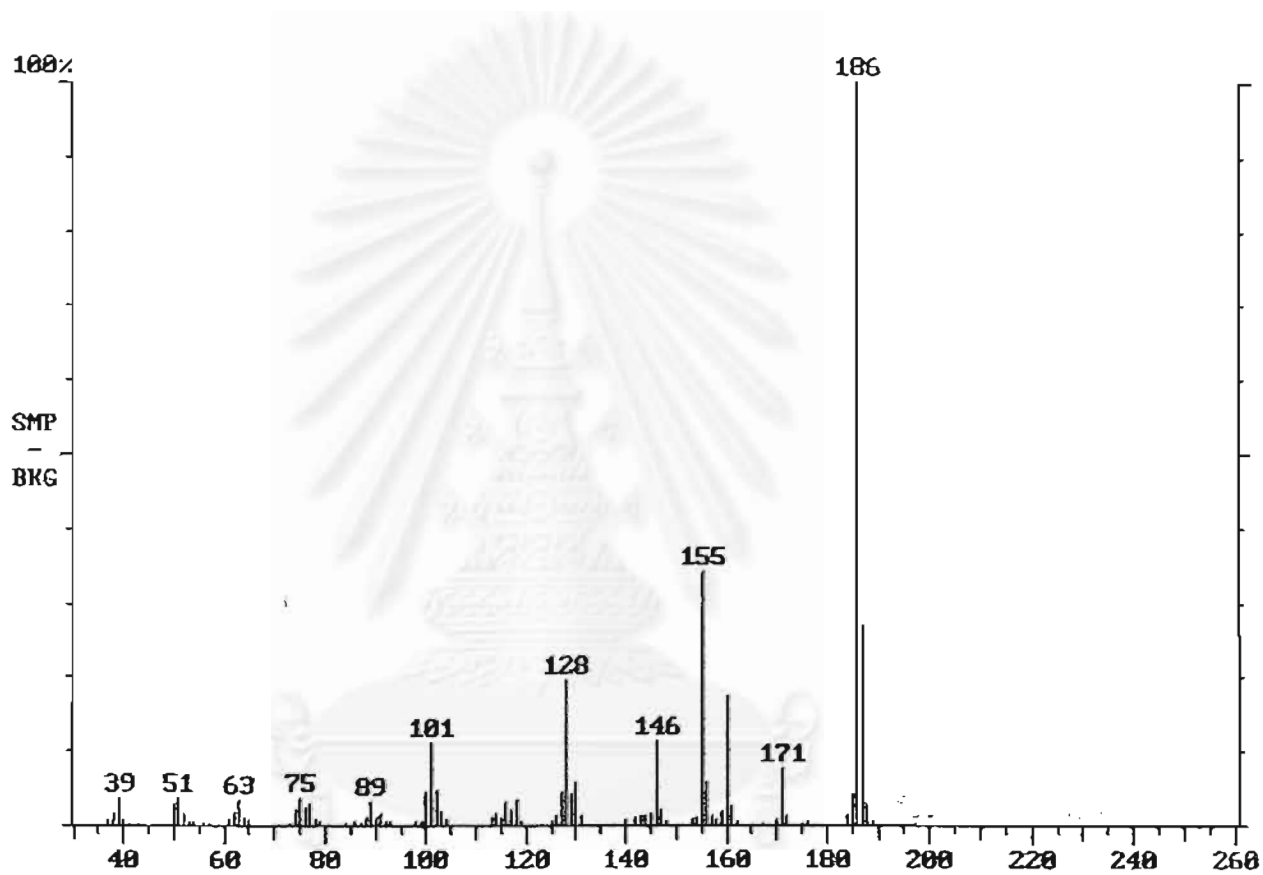
Fig.3.28 The mass spectrum of Compound 8
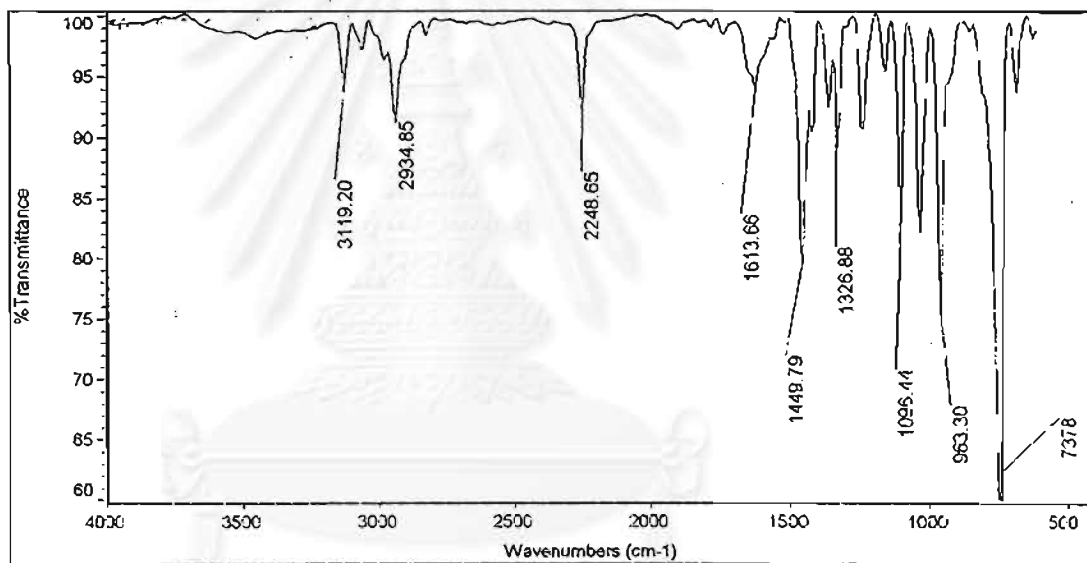
Fig. 3.29 The IR spectrum of Compound **8**
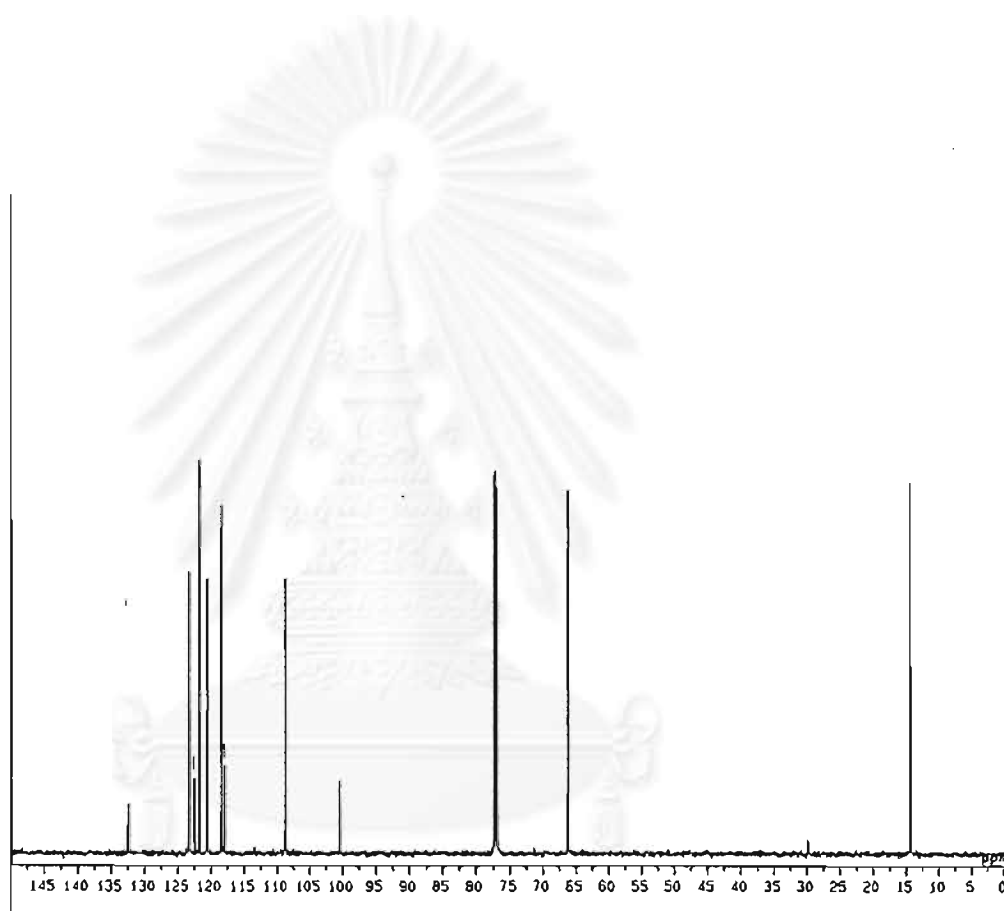
Fig. 3.30 The ^{13}C NMR spectrum of Compound **8**
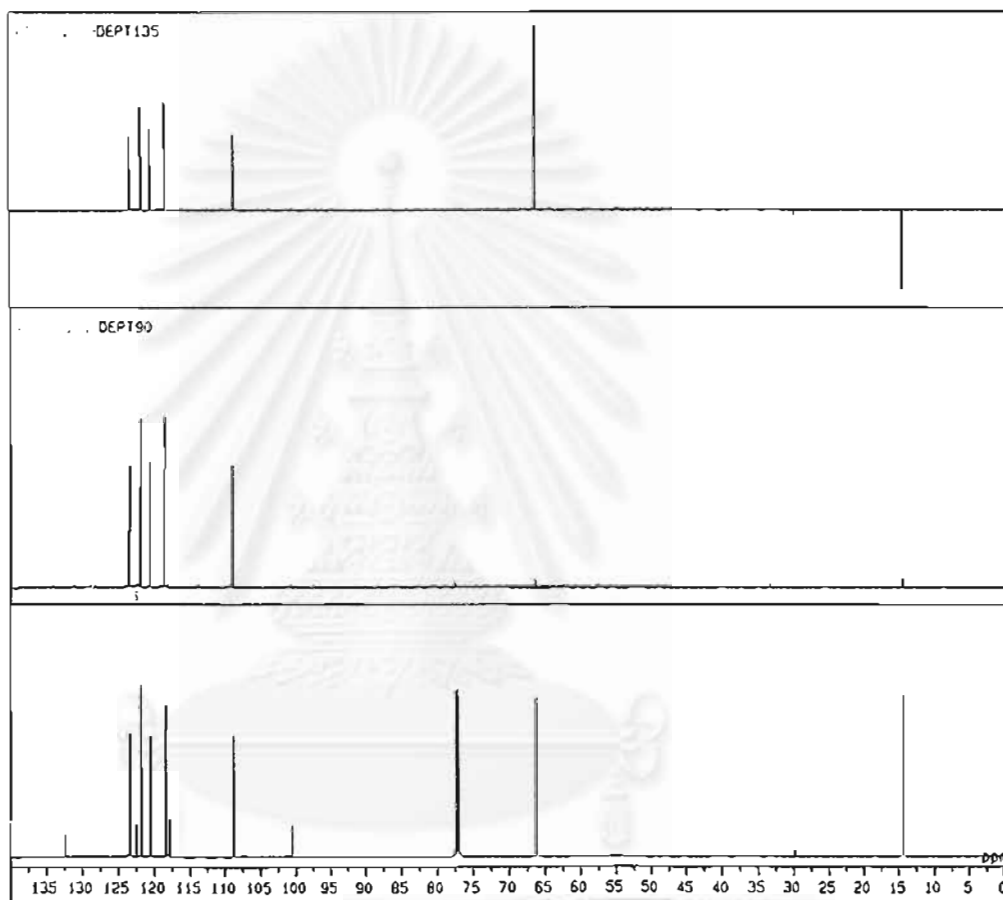
Fig. 3.31 The DEPT 90 and 135 spectra of Compound **8**
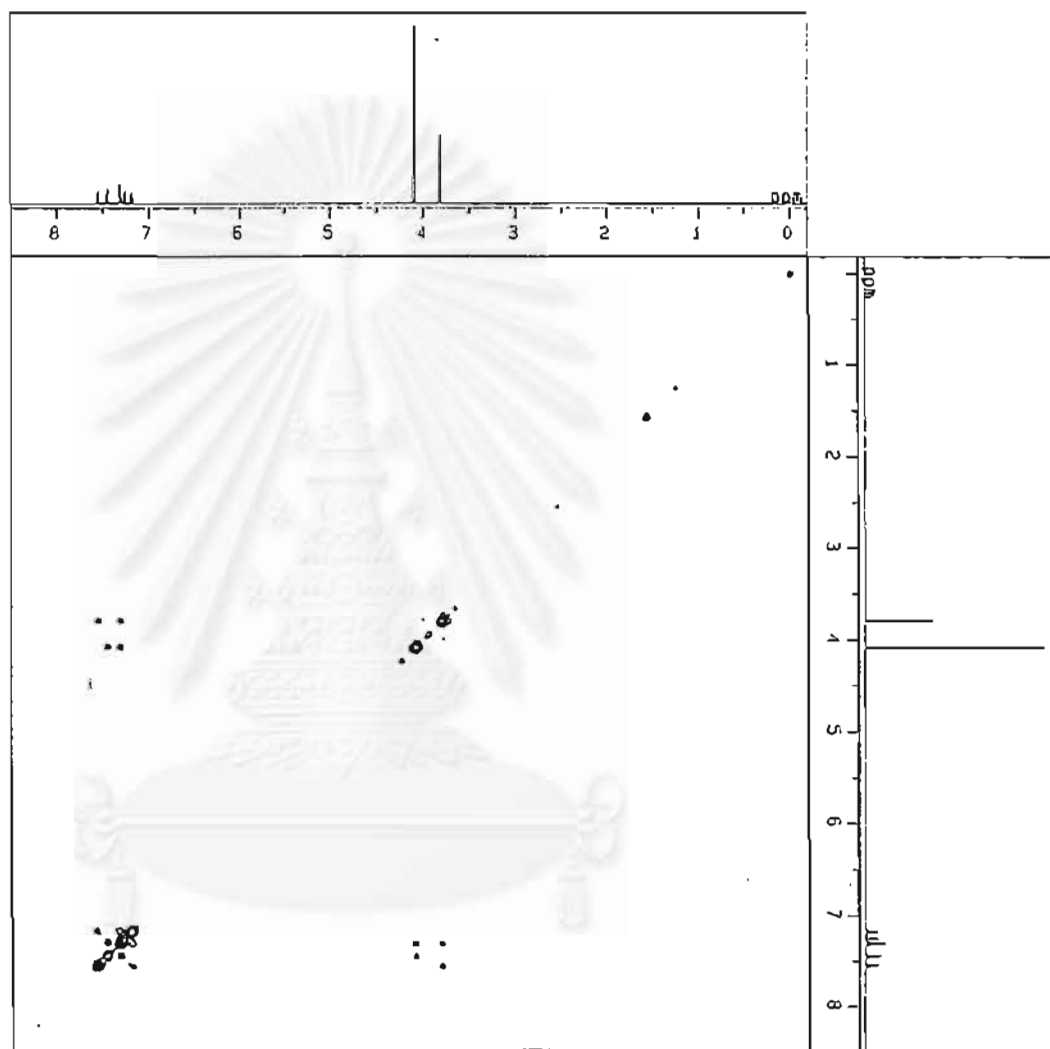
Fig. 3.32 The NOESY spectrum of Compound 8
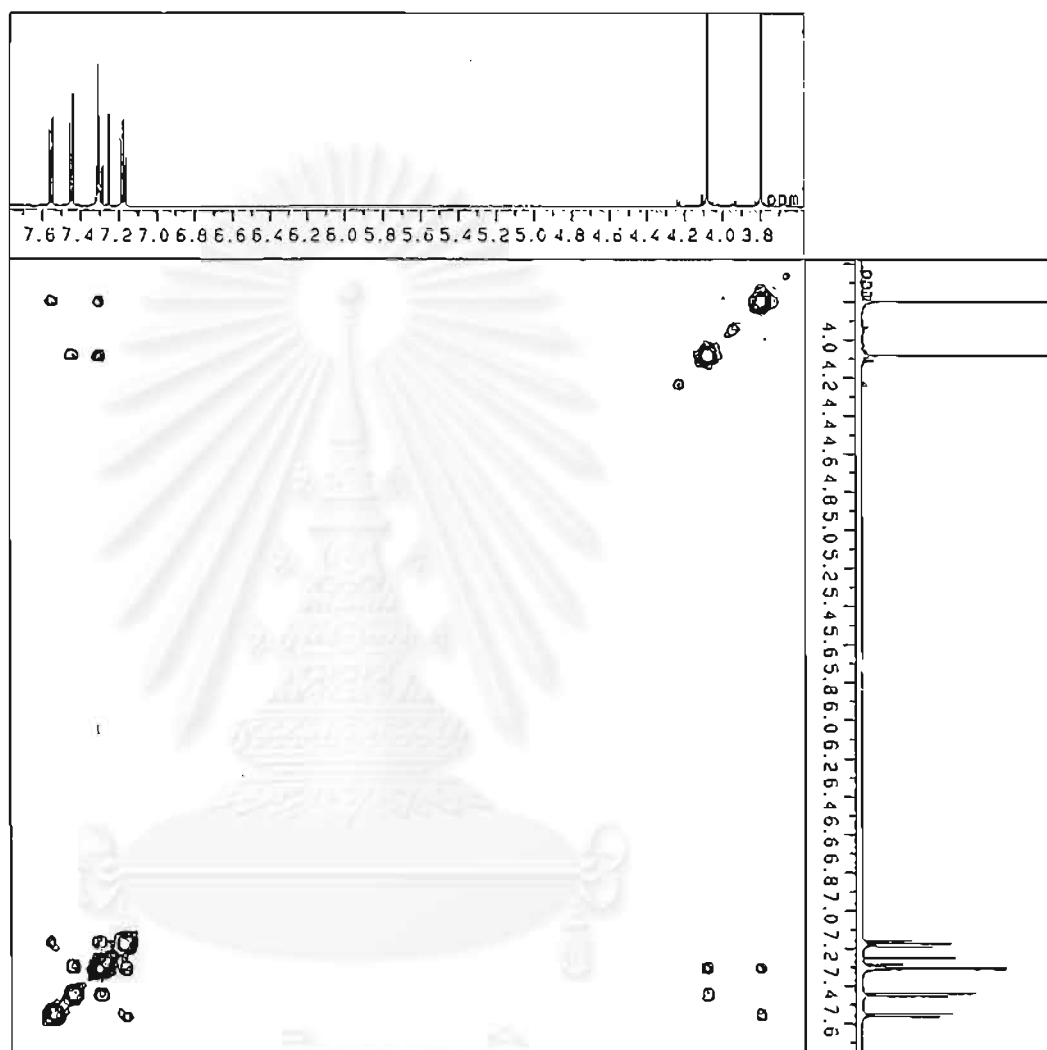
Fig. 3.33 The expanded NOESY spectrum of Compound **8**
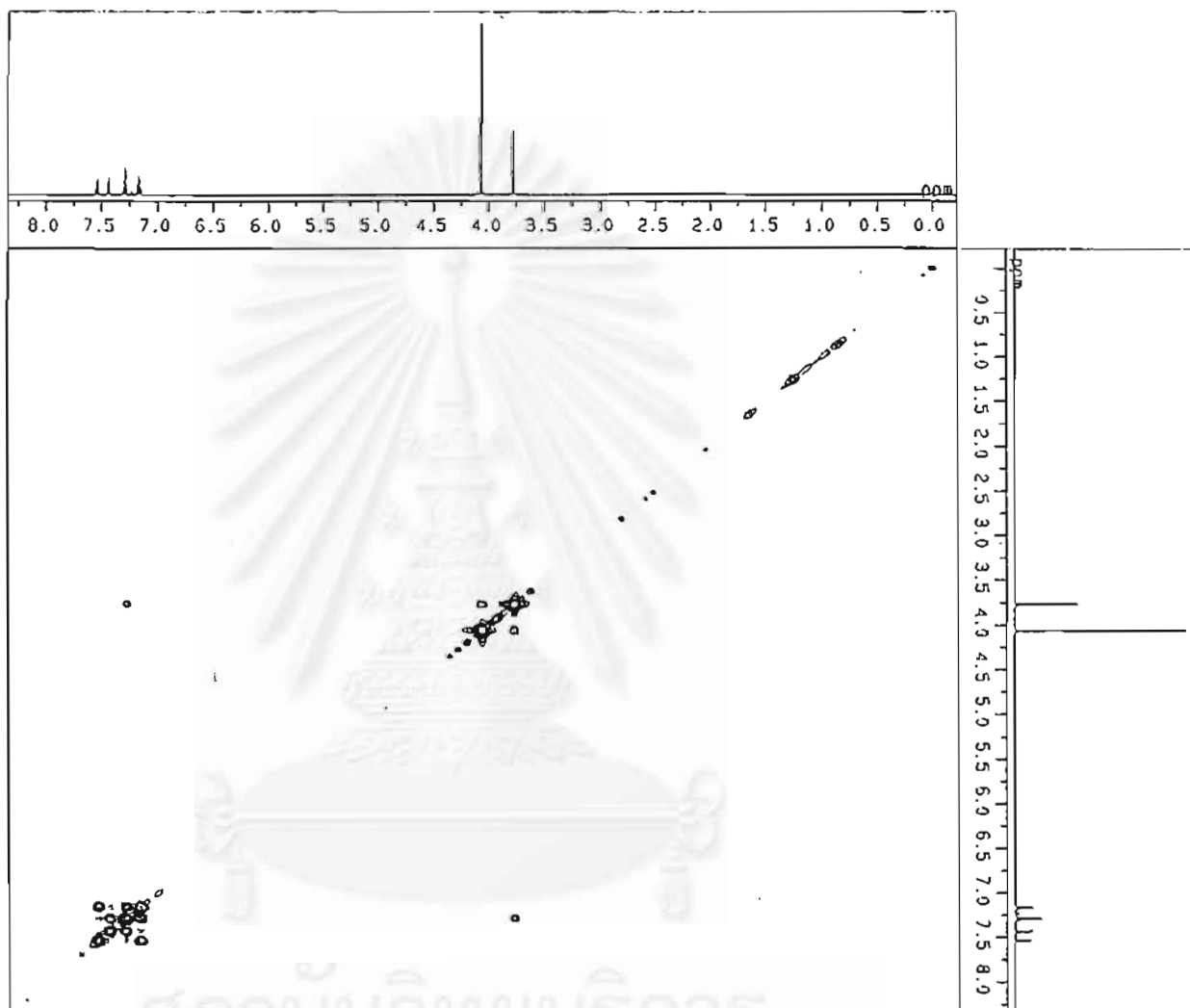
Fig. 3.34 ^{1}H-^{1}H COSY spectrum of Compound **8**
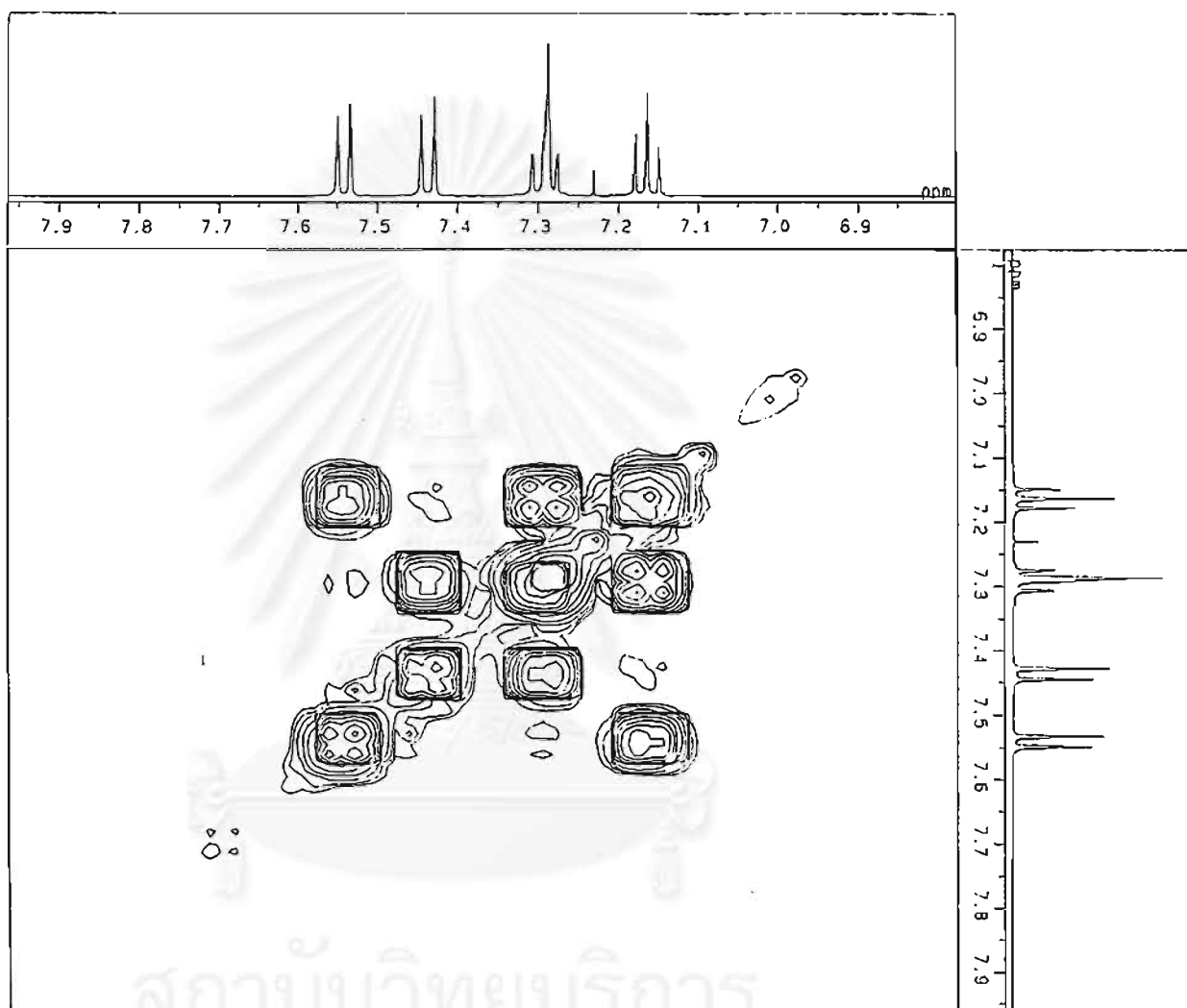
Fig. 3.35 The expanded ^{1}H-^{1}H COSY spectrum of Compound 8
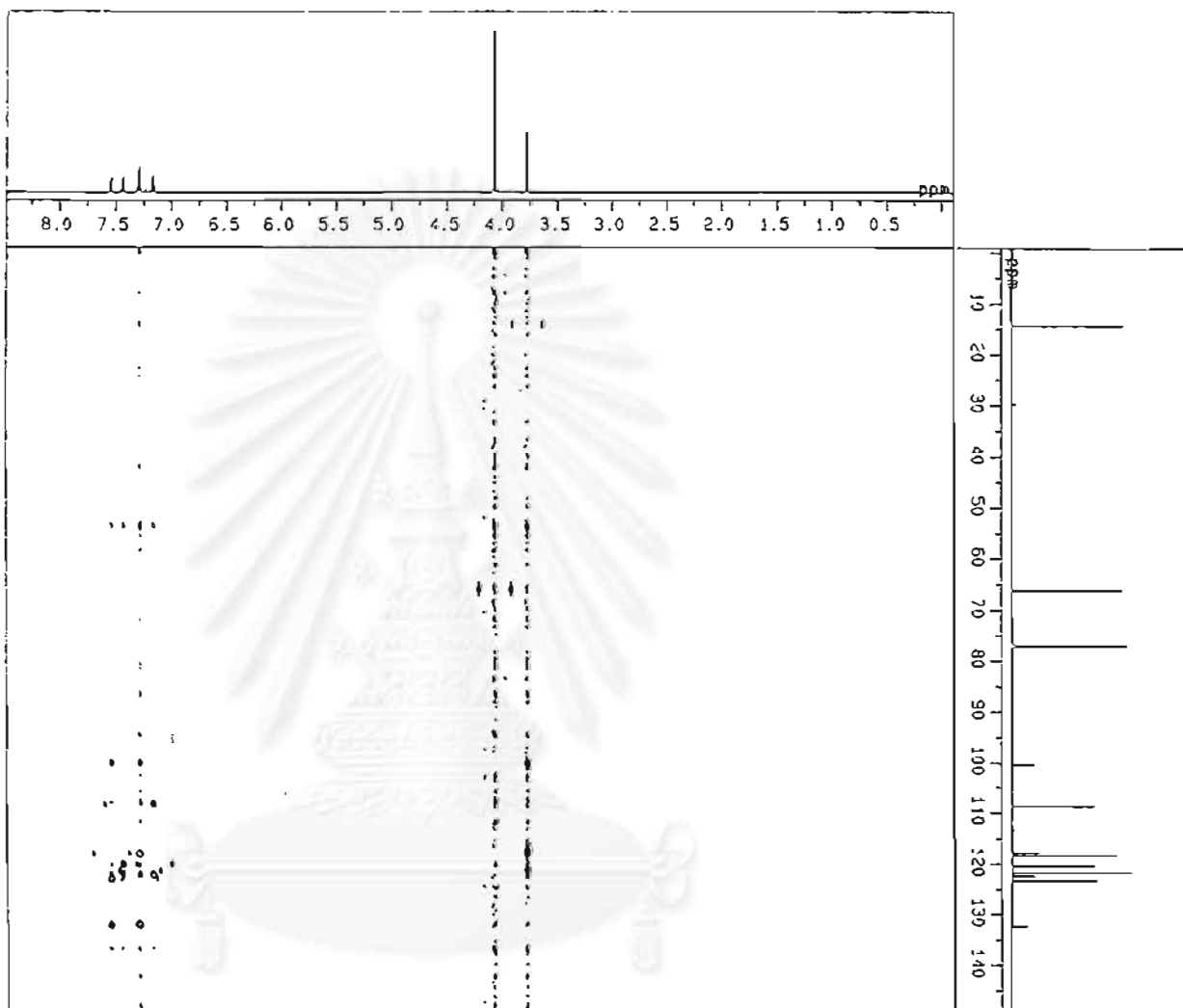
Fig. 3.36 The HMBC spectrum of Compound **8**
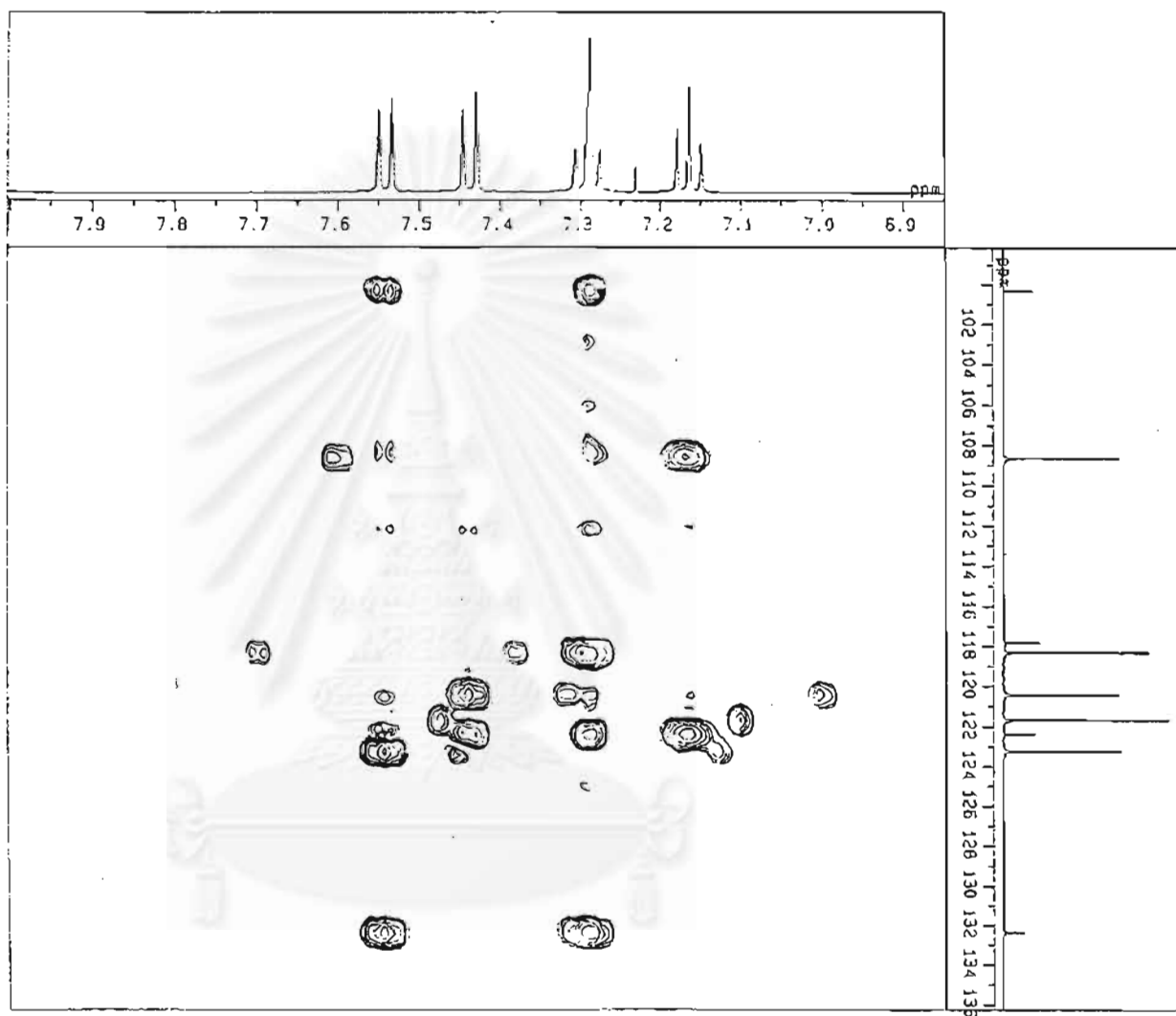
Fig. 3.37 The expanded HMBC spectrum of Compound **8**

Fig. 3.38 The HMQC spectrum of Compound 8
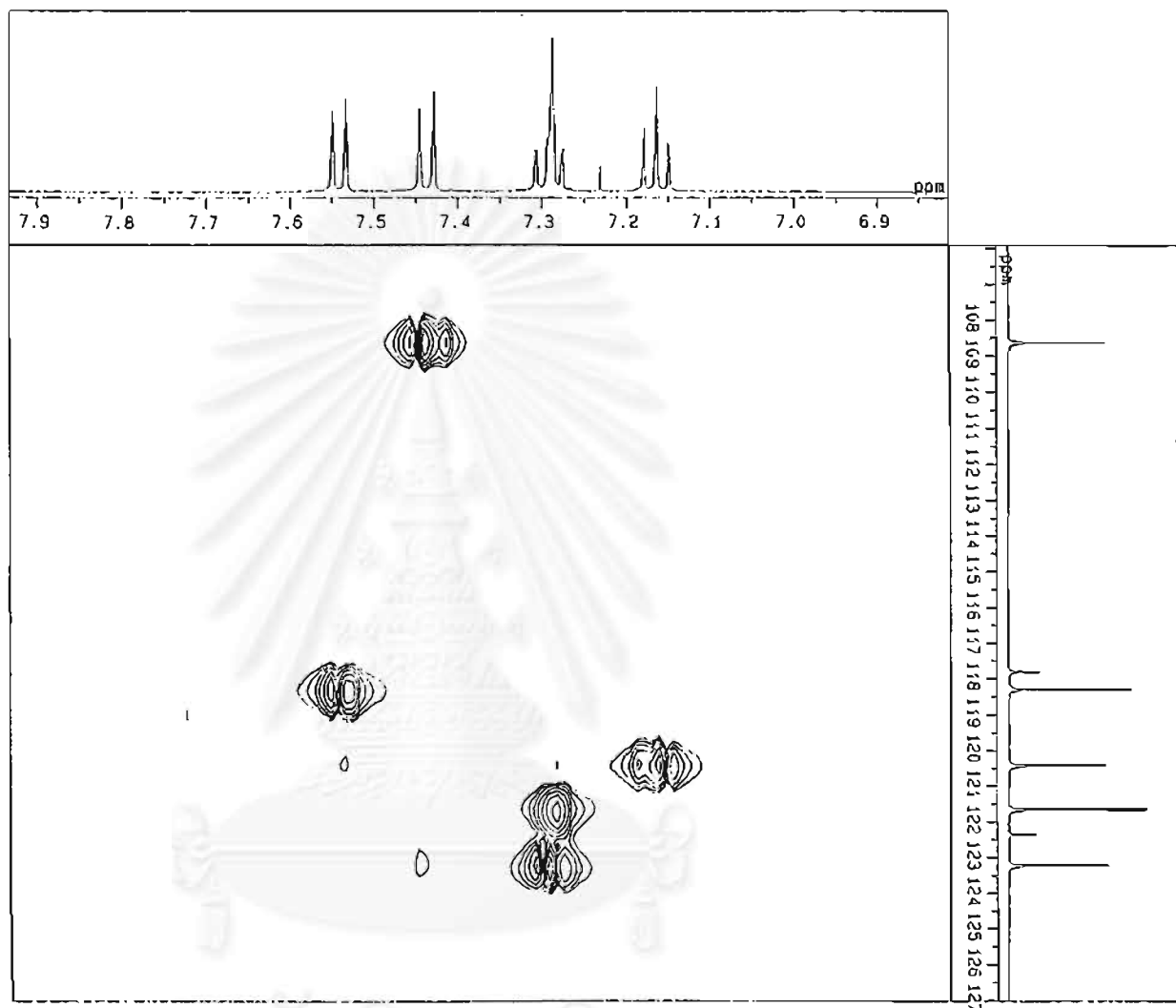
Fig. 3.39 The expanded HMQC spectrum of Compound 8

3.4.9 Physical properties and structural elucidation of Compound 9

Compound **9** was an orange viscous liquid obtained from both the dichloromethane crude extract and the ethyl acetate crude extract of the roots, (108 mg, 0.27 % yield w/w and 25 mg, 0.17% yield w/w respectively). It had R_f of 0.53 in 50% $CHCl_3$/EtOAc and could be dissolved in chloroform and ethyl acetate.

From the IR spectrum (Fig. 3.42), the presence of a carbonyl group was deduced. This carbonyl group could be an aldehyde due to the characteristic C-H stretching at 2930 and 2845 cm^{-1}. Another conspicuous functional group was the hydroxy group (v_{max} 3375 cm^{-1}) which could be identified from the IR spectrum (Fig. 3.42) as well. In addition, there was the absorption band at 1028 cm^{-1} of ring breathing of a furan. The mass spectrum indicated the presence of aldehyde and hydroxy group : 125 $[M-H]^+$ for an aldehyde group and m/z 109 $[M-OH]^+$ for a hydroxy group. The molecular formula could be deduced ($C_6H_6O_3$, MW= 126) from the NMR spectral evidence and the molecular peak at m/z 126.

The ^{13}C-NMR spectrum (Fig.3.41) illustrated the most downfield tertiary carbon of an aldehyde at δ 177.7 ppm and a methylene carbon, which directly attached to an oxygen atom at δ 64.6 ppm. Thus, there were two substituents attached to the furan ring. One group must be an aldehyde and the other should be a hydroxymethyl group ($HOCH_2$-). The furan skeleton possessed four carbon signals at δ 111.9, 121.9, 152.5, and 158.2. The ^{1}H-NMR spectrum (Fig. 3.40) exhibited signals corresponding to those of assigned carbons such as the methylene protons resonated at 4.72 (2H, s). Furano protons were observed at δ 6.25 (1H, d, J= 3.7 Hz) and 7.22 (1H, d, J=3.4 Hz). The magnitude of the coupling constant, 3.4- 3.7 Hz , is consistent with the coupling of H-3 and H-4. The substituted furan as mentioned above, therefore, was a 2,5-disubstituted furan. All spectroscopic evidences supported that Compound **9** was 5-hydroxymethylfurfuraldehyde or 5-HMF, which has been previously reported.[28, 29]

Its structure is shown below.
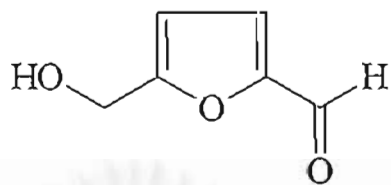
Structure of Compound 9
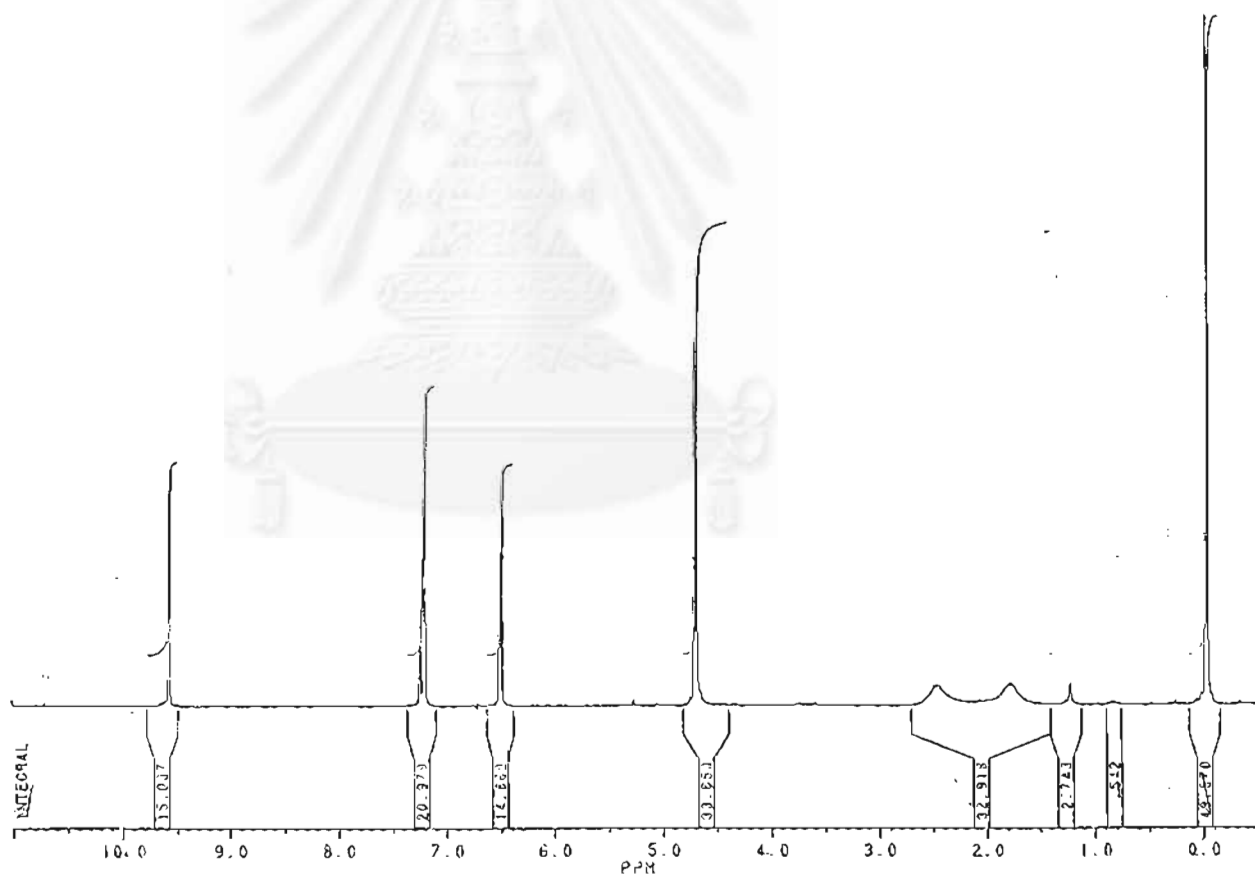

Fig. 3.40 The ^{1}H NMR spectrum of Compound **9**
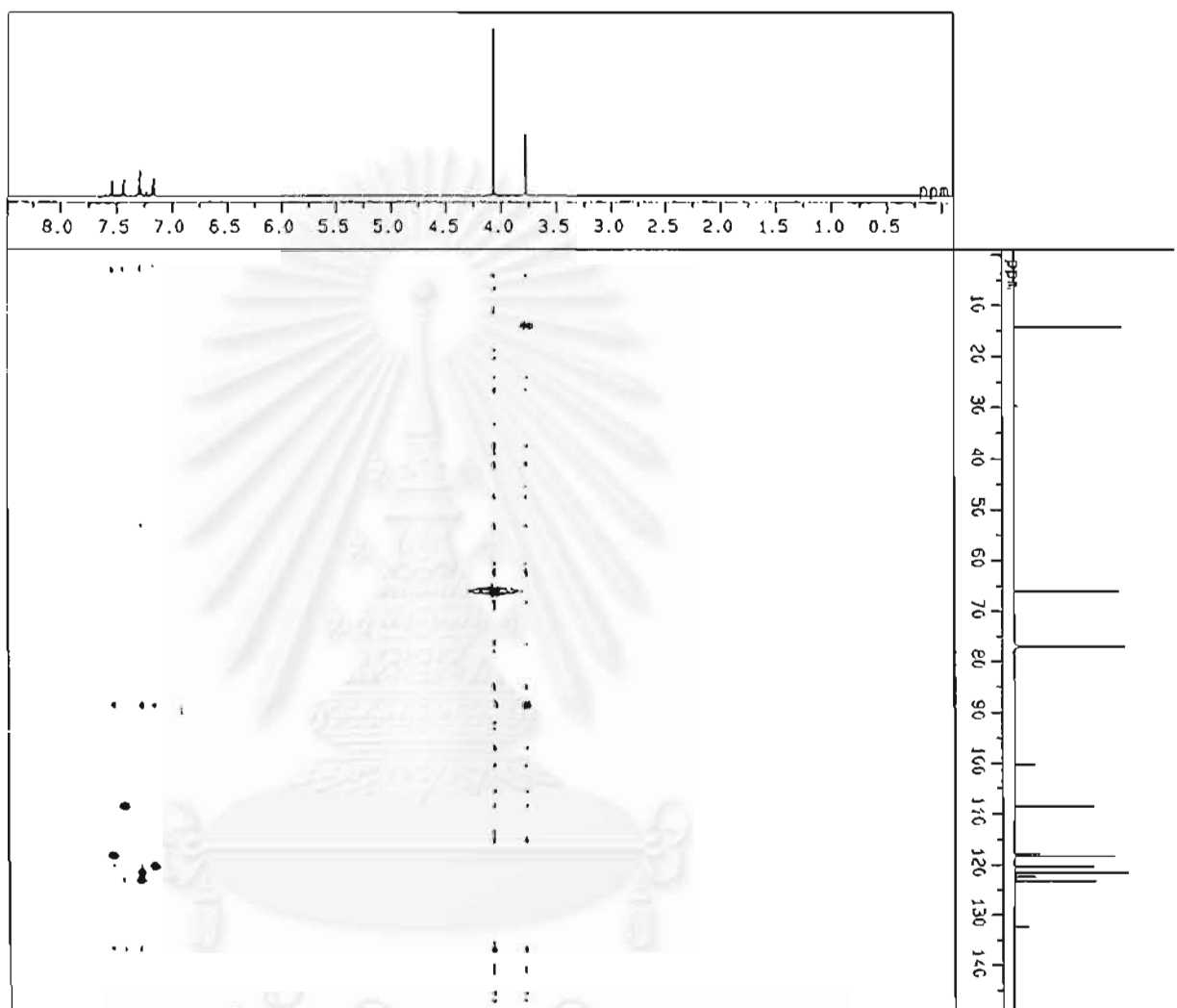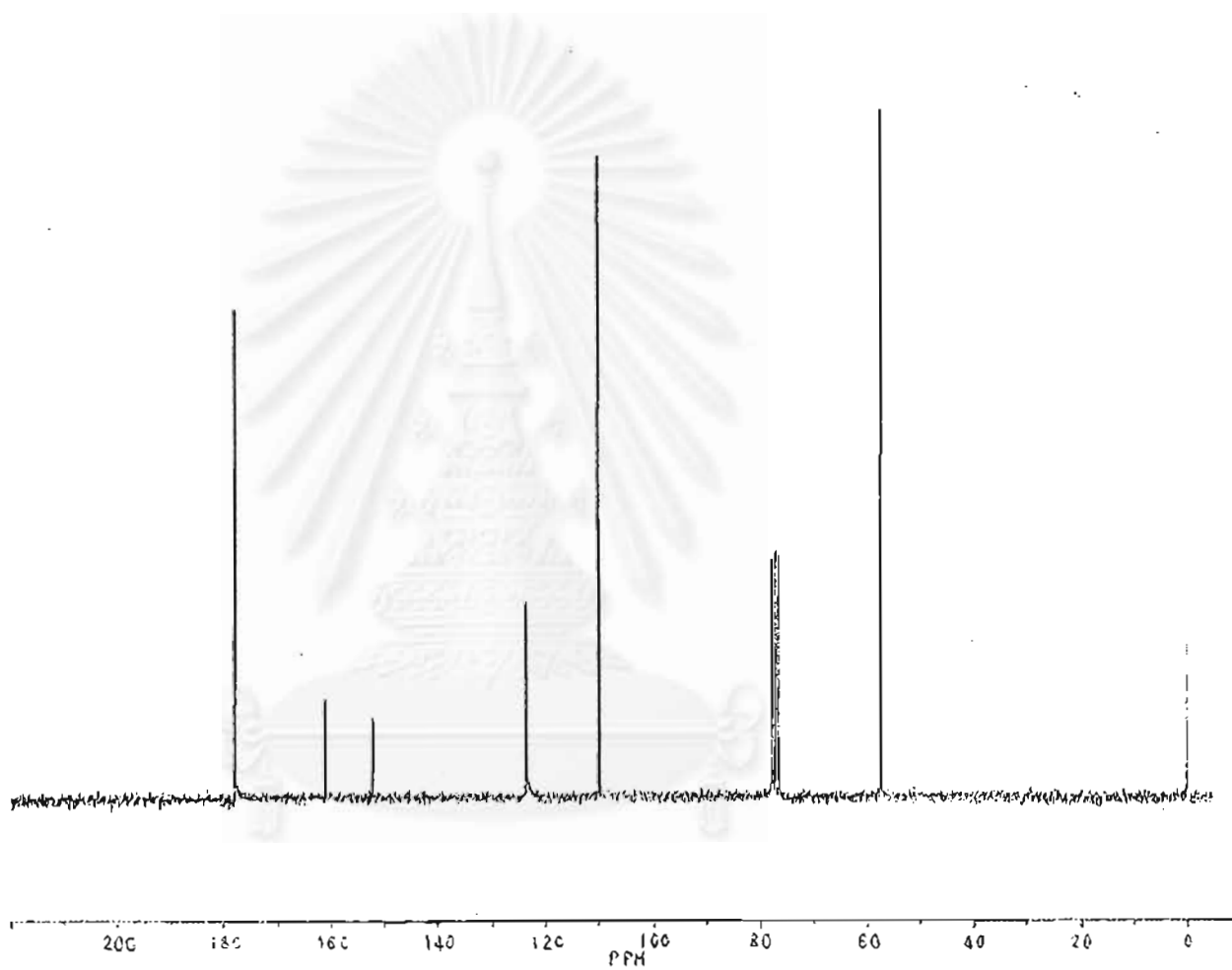
Fig. 3.41 The ^{13}C NMR spectrum of Compound **9**
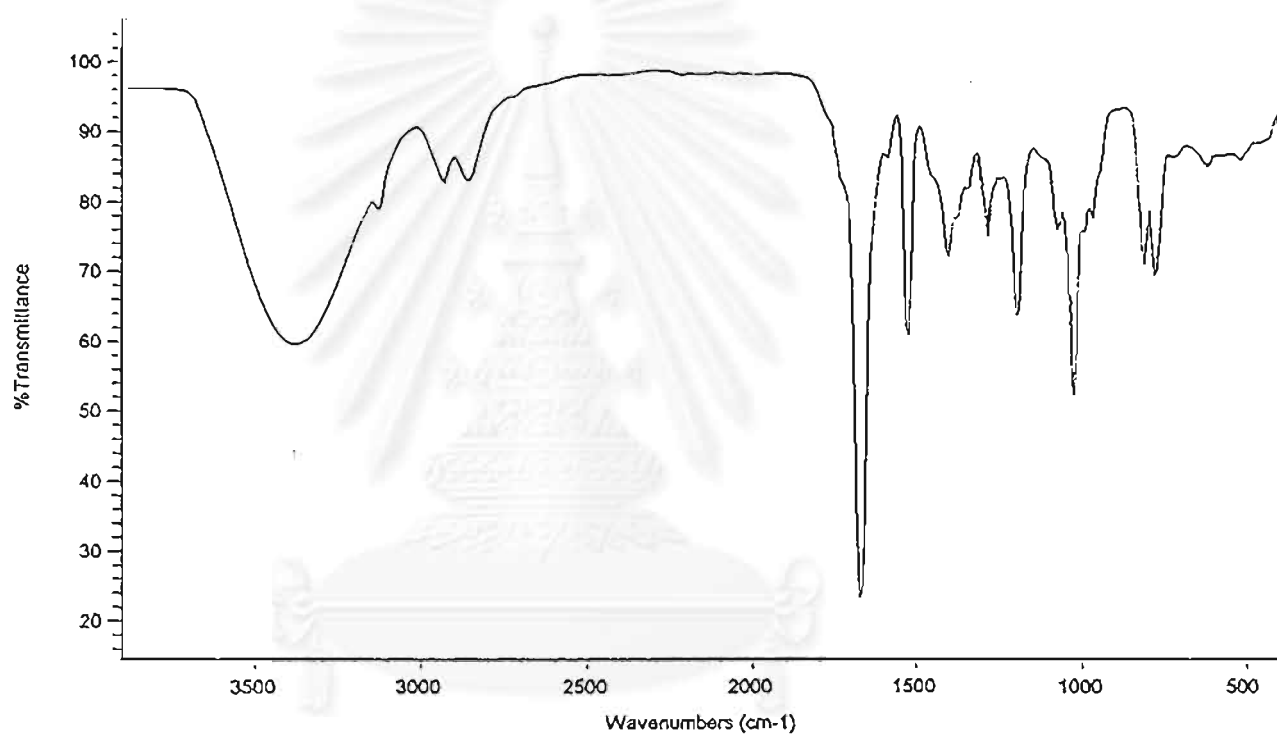
Fig. 3.42 The IR spectrum of Compound 9
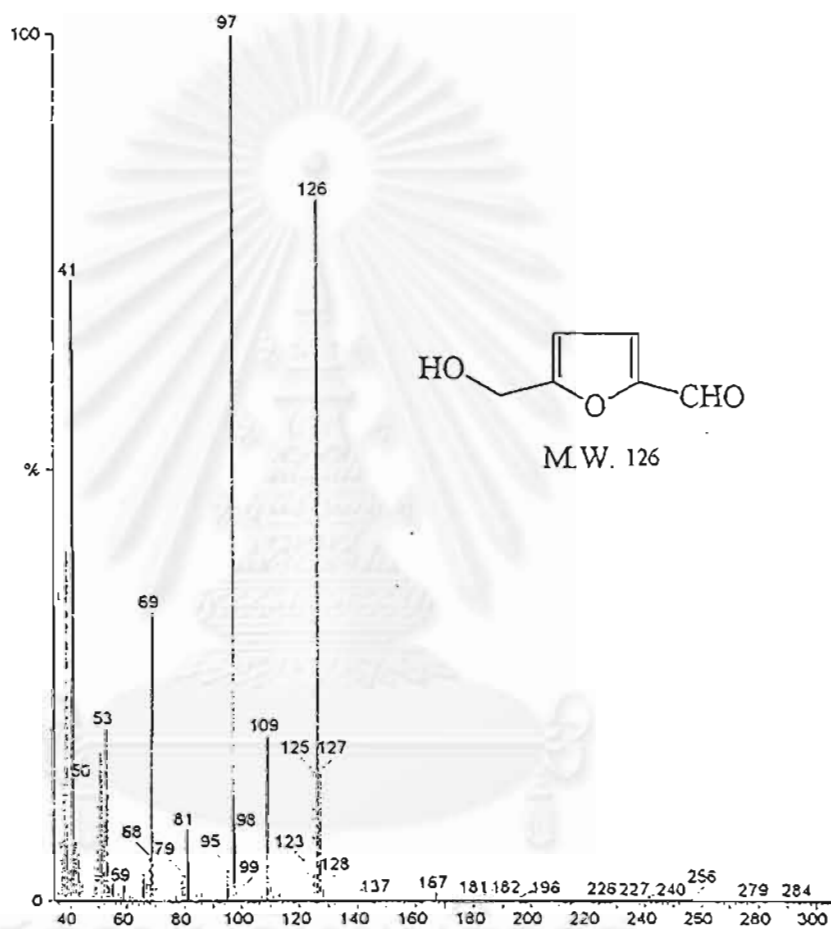
Fig. 3.43 The mass spectrum of Compound 9

3.4.10 Physical properties and structural elucidation of Compound 10

In Fr.No. 10 of the dichloromethane and Fr.No. 5 of the ethyl acetate crude extracts, Compound 10 was obtained as a white powder. It was recrystallized several times from hot 100% MeOH (20 mg, 0.05 % yield of dichloromethane extract and 125 mg, 0.83 % yield in ethyl acetate extract, m.p. 266-268 °C. This compound was soluble in hot MeOH and has an R_f value of 0.67 in 3:7 MeOH/CH$_2$Cl$_2$ solvent system.

Absorption bands of the IR spectrum are shown in the Table 3.19.

Table 3.19 Some important IR absorption bands of Compound 10

absorption band (cm^{-1})	band type	characteristic
3500-3200	s	O-H stretching of C-OH
2390	s	O-H stretching of CH$_3$, CH$_2$
1640	w	C=C stretching of alkene
1470	m	C-H bending of CH$_3$, CH$_2$
1383	m	C-H bending of –CH-(CH$_3$)$_2$
1250	w	C-O stretching
1160	m	C-O stretching
1075	s	C-O stretching
1019	s	C-O stretching

The IR spectrum (Fig. 3.44) showed absorption bands which are typical of a glycoside: broad absorption band at 3500-3200 cm^{-1} of hydroxy group, at 1250, 1160, 1075, and 1019 which are C-O stretching of hydroxy groups and at 890 cm^{-1}, which is the signal of anomeric axial proton of a β-sugar.

Six carbon signals were in the ^{13}C NMR spectrum (Fig. 3.46); δ 100.8, 76.9, 76.7, 73.4, 70.1 and 61.1 ppm, which were very similar to those of D-glucose.[26] An anomeric proton at δ 4.23 (1H, d, J= 7.6 Hz) could be the signal of β-anomeric

proton of this sugar while a group of multiplet signals at δ 4.40-5.40 ppm were signals of sugar moiety. Also, by comparing the ^{13}C-NMR chemical shifts of known stigmasteryl-3-O-β-D-glucopyranoside, they were closely equivalent (Table 3.20).

Table 3.20 ^{13}C-NMR chemical shifts of aglycone of Compound 10 and those of stigmasteryl-3-O-β-D-glucopyranoside[30]

carbon Position	Compound 10	stigmasteryl-3-O-β-D-glucopyranoside
1	36.8	37.4
2	29.3	31.7
3	70.1	71.8
4	41.8	42.4
5	140.4	140.0
6	121.2	121.7
7	31.4	31.9
8	31.4	31.9
9	49.6	50.3
10	36.2	36.6
11	22.6	21.1
12	38.3	39.8
13	41.8	42.4
14	56.2	57.0
15	23.9	24.4
16	28.7	28.9
17	55.4	56.0
18	11.8	12.2
19	19.7	19.4
20	40.8	40.5
21	20.6	21.1
22	138.5	138.4

Table 3.20 (cont.)

23	128.8	129.4
24	51.4	51.3
25	33.3	31.9
26	19.1	19.0
27	20.6	21.1
28	25.4	25.4
29	11.8	12.0

It can be summarized that Compound **10** must be a steroid glycoside, stigmasteryl-3-O-β-D-glucopyranoside. The structure of this compound is shown below.
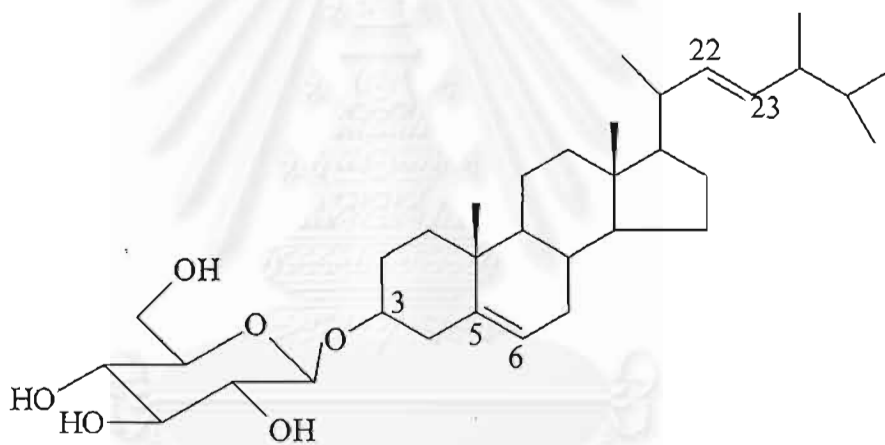
Structure of Compound 10
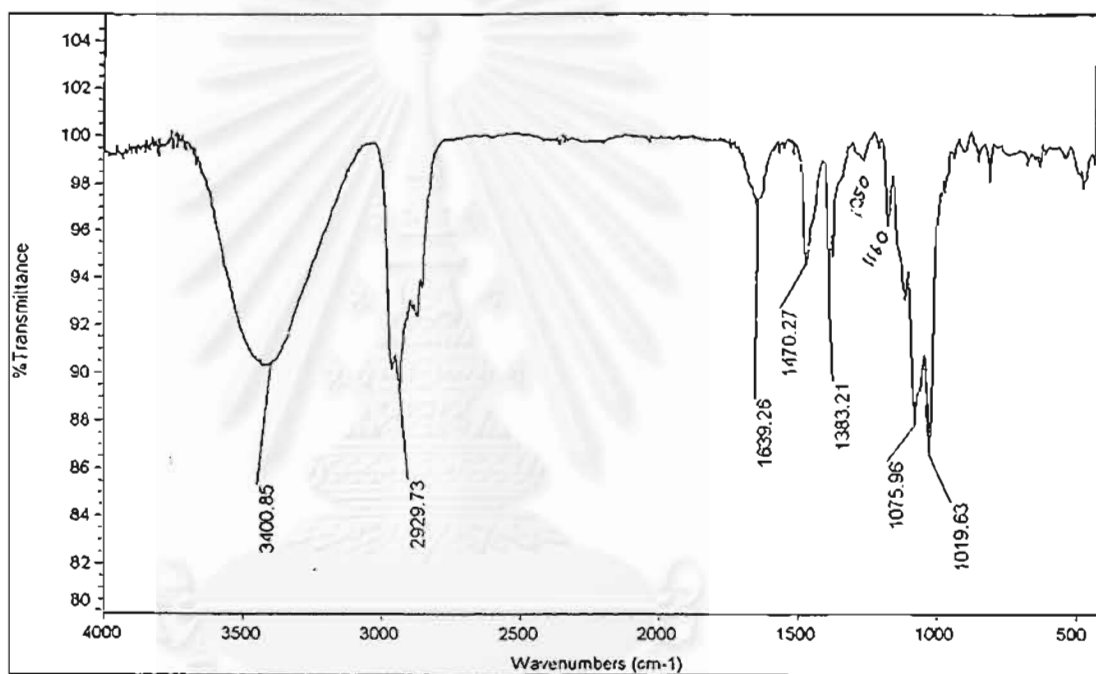
Fig. 3.44 The IR spectrum of Compound **10**
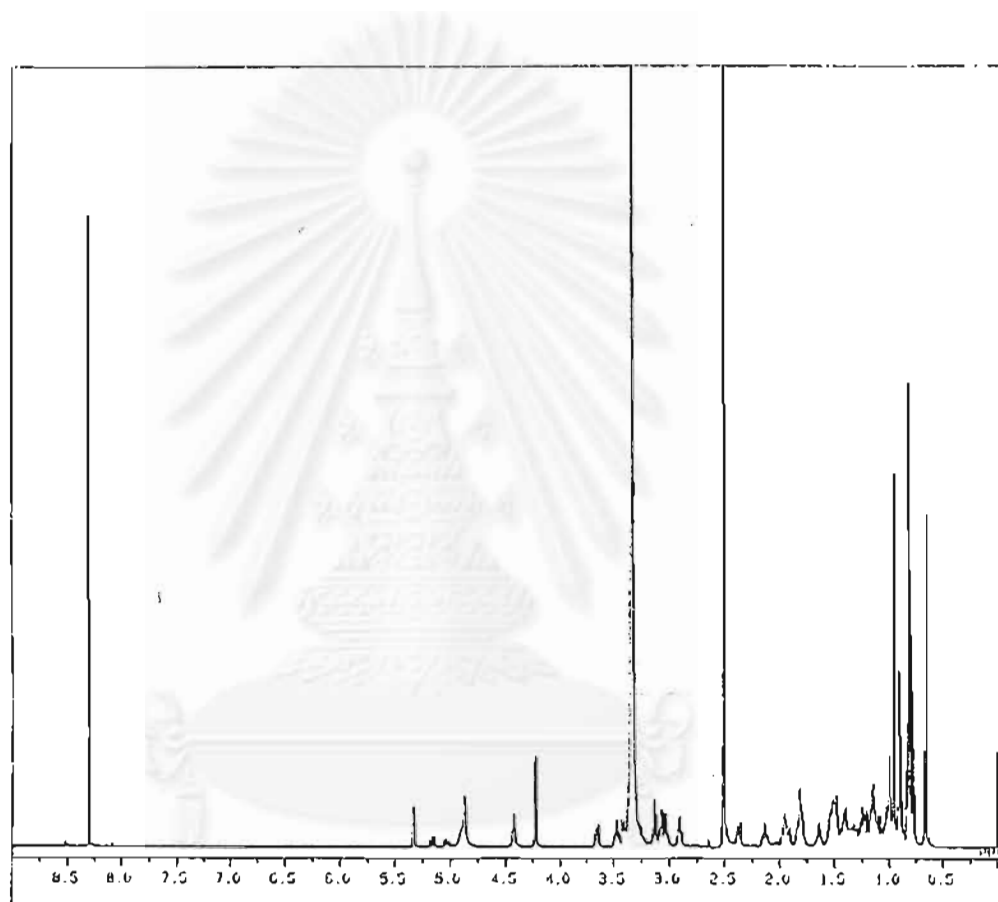
Fig. 3.45 The ^{1}H NMR spectrum of Compound **10**
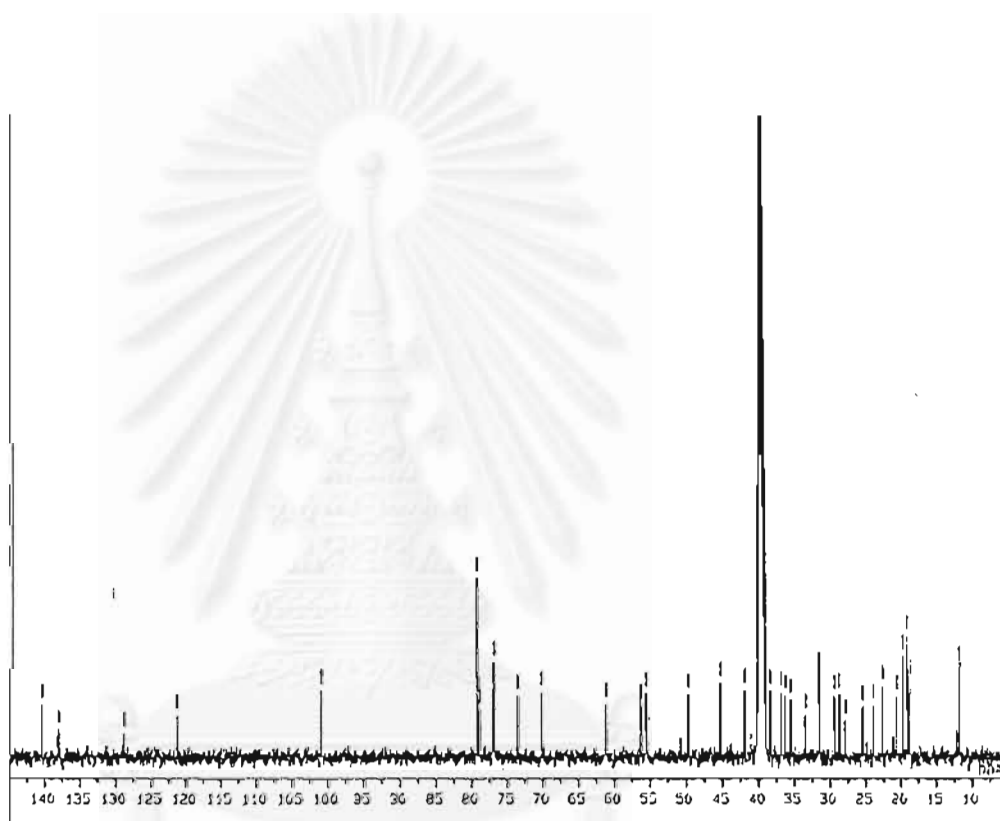
Fig. 3.46 The ^{13}C NMR spectrum of Compound **10**
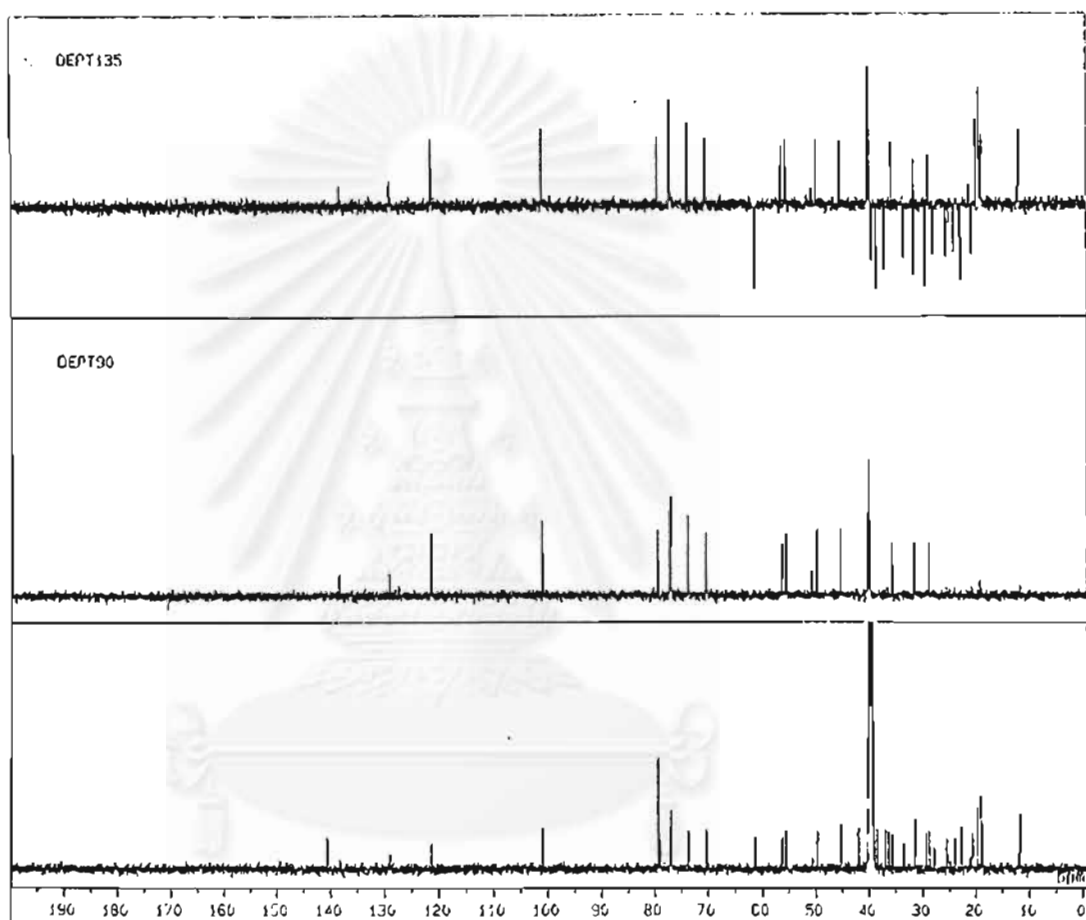
Fig. 3.47 The DEPT 90 and 135 spectra of Compound **10**

3.5 The results of biological activities of isolated compounds

3.5.1 The BSCLT of isolated compounds

Following the preliminary cytotoxic screening test, hexane crude extract of leaves gave high activity and ethanolic crude extract of roots gave medium activity against brine shrimp (*Artemia salina* Linn.). Both of them were chosen for further investigation of bioactive compounds.The isolated compounds were retested for the cytotoxic against brine shrimp. The result is displayed in Table 3.21.

Table 3.21 The result of brine shrimp lethality cytotoxicity test of isolated compounds

Compounds	LC_{50} (µg/ml)	activity
Compound 4	265.45	low activity
Compound 6	201.06	low activity
Compound 7	0.09	high activity
Compound 8	9.24	high activity
Compound 9	240.33	low activity
Compound 10	2816.60	no activity

From the result above, both Compounds 7 and 8 showed significant cytotoxicity against brine shrimp (*Artemia salina* Linn.).

3.5.2 Antioxidant activity

After developed by traditional method, TLC was sprayed with standard reagents (DDPH for free radical scavenging activity and β-carotene for β-carotene bleaching activity). The results are displayed in the Table 3.22.

Table 3.22 The result as antioxidant of isolated compounds

Compounds	Result	
	free radical scavenging	β-carotene bleaching
Compound 1	-	-
Compound 2	-	-
Compound 3	-	-
Compound 4	√	√
Compound 5	-	-
Compound 6	-	√
Compound 7	√	?
Compound 8	√	?
Compound 9	-	-
Compound 10	-	-

Compounds 4, 7 and 8 showed the positive free radical scavenging antioxidant activity. On the prevention of β-carotene bleaching activity, Compounds 4 and 6 showed the clear positive result, but Compounds 7 and 8 cannot be determined because of the ambiguous result.

3.5.3 Antibacterial activity

At dose 10 ppm of pure isolated compounds, there was no inhibitory effect of those compounds on bacteria strain.

3.6 Biological activity studies of isolated compounds

From the preliminary bioactivities of the leaf extracts which were separated by procedure in Scheme 2.1, hexane crude extract exhibited high cytotoxic lethality on brine shrimp (*Artemia salina* Linn.) (LC_{50} 3.44 μg/ml) and also has inhibitory activity on various types of bacteria (Table 3.3) which led to investigate the bioactive compounds from this crude extracts. The isolation of the hexane crude extract yielded three triterpenoids: taraxerone, taraxerol, and unidentified triterpenoid I. There are several reasons to support interesting of root extracts to be investigated for chemical constituents: the history as a Thai herbal medicine, the presence of alkaloids by chemical screening and the interesting bioactivities of this crude extract on brine shrimp (LC_{50} 57.96 μg/ml) and plant growth inhibition (Table 3.4). Seven compounds were obtained from the roots: unidentified triterpenoid II, stigmasterol, 1H-indole-3-carboxaldehyde, 1-methoxy-indole-3-carboxaldehyde, 1-methoxy-indole-3-acetonitrile, 5-hydroxymethylfurfuraldehyde and stimasteryl-3-O-β-D-glucopyranoside. All compounds isolated from this plant in this research were reported for the first time.

Compound 1 (taraxerone), Compound 2 (taraxerol) and Compound 3 (triterpenoid I) were isolated from the leaves. They did not show significant cytotoxic lethality on brine shrimp and had no free radical scavenging activity. In general, triterpenoidal compounds are always used as anti-inflammatory agent. From the presence of triterpenoid as the major component in the leaves, we may utilize the leaves directly as an anti-inflammatory agent like the roots.

Compound 4 was isolated from the roots and has not been elucidated for its structure. From the bioassay results, Compound 4 showed the positive result in both antioxidant activity tests, which might lead to being antioxidant substance. However, this compound should be tested for further specific antioxidative bioassay and elucidated for its structure.

Compound **5** (stigmasterol), and Compound **10** (its glycoside, stigmasteryl-3-O-β-D-glucopyranoside) were separated from the roots. They did not exhibit interesting activities on brine shrimp and free radical scavenging bioassay. They were the steroidal compounds which were the major components of the roots. This perhaps indicated why the roots were utilized as an anti-inflammatory agent.

Compound **6** was isolated from the roots of *A. sarmentosa* and characterized its structure as 1H-indole-3-carboxaldehyde. From literature surveys, this compound has been isolated from a marine sponge, *Halichondria sp.* and had antifungal activity against *Mortierella ramannianus*. [31] When treated with nitrite at pH 3, indole-3-carboxaldehyde and seven other indole compounds, became mutagenic on 3 tester strains (*Samonella typhimurium* TA 98 and TA 100 and Escherichia coli WP$_2$) without metabolic activation system (S$_9$ mix). Its mutagenicity was decreased by the addition of S$_9$ mix.[32] Sunscreens containing indole-3-carboxaldehyde prevent UV-induced peroxidation of skin lipids show skin-lightening effect and prevent sunburn and rough skin. The IC$_{50}$ value of this compound against UVB-induced peroxidation of rabbit erythrocyte membrane was 29.5 μg/ml (60 μg/ml of α- tocopherol). Polyoxyethylene hydrogenated caster oil 1.0, ethanol 15.0, citric acid 0.1, sodium citrate 0.3, 1,3-butylene glycol 4.0 indole-3-carboxaldehyde 0.05 wt.%, anticeptic, perfume and water balance have been mixed to give a sunscreen lotion.[33] From the bioassay result, it exhibited low cytotoxic lethality on brine shrimp. It can prevent UV-induced peroxidation as described above, according with the positive result in prevention of β-carotene bleaching bioassay.

Compound **7** was separated from the roots and characterized as 1-methoxy-indole-3-carboxaldehyde, which contained the unusual N-methoxy in its structure.This compound, 1-methoxy-3-indole-carboaldehyde, has been obtained from natural source and synthesis. [34, 35] There was a report of isolation of this compound from *Brassica oleracea*.[36] In a biological study, this 1,3-disubstituted indole was found to be a more potent inducer of monooxygenase activity than any of the 3-substitued indole tested.[37] This result accorded with the higher cytotoxicity (LC$_{50}$ 0.09 μg/ml) on brine shrimp of Compound **7** than those of 3-substituted Compound **6**

(LC$_{50}$ 201.06 µg/ml). It also showed the positive result in the free radical scavenging activity.

Compound **8** was isolated from the roots and identified as 1-methoxy-indole-3-acetonitrile. From the literature surveys, this compound was isolated not only from microorganism but also from higher plant, clubroots of Chinese cabbage and it showed the slight activity on the *Avena* coleoptile straight growth.[38] From the bioassay results, Compound **8** displayed the high cytotoxic lethality on brine shrimp (LC$_{50}$ 9.24 µg/ml), which might be further tested for anticancer activity. Moreover, it showed the positive result in free radical scavenging activity.

Compound **9** was separated from the roots of *A. sarmentosa* and its structure was established as 5-hydroxymethylfurfuraldehyde (5-HMF). 5-HMF has been isolated from several plants[29,39,40,41] and also can be commercially prepared by acid dehydration from various kinds of sugar, particular from hexoses. 5-HMF was of interest owing to a number of their pharmacological activities. The products of reactions between 5-HMF and monohydroxy-1,4-naphthoquinones were used as sunburn-prevention containing cosmetics.[42] Moreover, it was active as an anthelmintic against *Clenorchia sinensis* (Chinese liver fluke). From the bioassay results, it did not show the interesting activity on both brine shrip and in free radical scavenging activity

All compounds have no inhibitory effect on bacteria at dose 10 ppm, which was the interesting point to continue testing for minimum inhibitory concentration on those bacteria.

Chapter IV
Conclusion

In the course of research work, the leaves and roots *of Azima sarmentosa* Benth are selected for investigating their chemical constituents and their bioactivities. The preliminary screening bioactivity of hexane crude extract of the leaves on brine shrimp cytotoxic lethality test (LC_{50} 3.44 µg/ml) and interesting antibacterial activity was the guide to continuing investigation of bioactive compounds from this crude extract. For the roots, there are the report of utilization as a Thai herbal medicine and the presence of alkaloids by chemical screening. The crude extracts of the roots also showed the interesting cytotoxic lethality on brine shrimp (LC_{50} 55.96 µg/ml) and plant growth inhibition. The hexane, the dichloromethane and the ethyl acetate crude extracts were selected to investigate bioactive compounds and study for their biological activities.

Chemical constituents of leaf and root extracts from *A. sarmentosa* Benth (Salvadoracae) were investigated. From leaf extract, there are three compounds isolated:

Compound **1, taraxerone**

Compound **2, taraxerol**

Compound **3, unidentified triterpenoid I**

and from root extract , there are seven compounds gained :

Compound **4, unidentified triterpenoid II**

Compound **5, stigmasterol**

Compound **6, 1H-indole-3-carboxaldehyde**

Compound **7, 1-methoxy-indole-3-carboxaldehyde**

Compound **8, 1-methoxy-indole-3-acetronitrile**

Compound **9, 5-hydroxymethylfurfuraldehyde**

Compound **10, stigmasteryl-3-O-β-D-glucopyranoside**

All compounds isolated were reported for the first time in this plant.

In the aspect of biological activities, Compounds **7** and **8** showed the significant cytotoxic lethality on brine shrimp (LC_{50} 0.09 and 9.24 µg/ml respectively).

Moreover, Compounds **4**, **7** and **8** gave the positive result as free radical scavenging and Compounds **4** and **6** gave the positive result in prevention of β- carotene bleaching antioxidants. However, there is no inhibitory effect of all isolated compounds on bacteria at dose 10 ppm.

Proposal for the future work

The discovery of compounds belonging to *A. sarmentosa* firstly reported in this thesis would be interesting for future investigation. The hexane crude of the leaves shows high cytotoxic lethality on brine shrimp, but triterpenoidal compounds isolated from this crude extract did not show any activity, so it is interesting to investigate bioactive compounds which have not been isolated from this crude extract. In root extract, although Compounds **4**, **6**, **7** and **8** possess the free radical scavenging antioxidant, they have to be tested in other specific antioxidative bioassays. Moreover, Compound 4 should be elucidated its structure which might lead to discovery of new-structure compound to be additional information about organic compounds. Compounds 7 and 8 exhibit the high cytotoxic lethality on brine shrimp (LC_{50} 0.09 and 9.24 μg/ml respectively) which might be tested further for anti-cancer activities or other specific bioassays. About the antibacterial activity of all isolated compounds tested, although there was no inhibitory effect at dose 10 ppm, they should be tested again for minimum inhibitory concentration (MIC). In addition, of both root and leaf extracts, there is major yield of polar-solvent extracts, therefore, in the aspect of searching for chemical constituents, polar solvent extracts might be investigated which may lead to discovery of new structure compounds or new useful drugs.

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