องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไลเพสของผลมะอึก *Solanum stramonifolium* Jacq.

นางสาว วาทินี จันมี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

CHEMICAL CONSTITUENTS AND LIPASE INHIBITORY ACTIVITY OF *Solanum stramonifolium* Jacq. FRUIT

Miss Watinee Chanmee

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2009

Thesis Title	CHEMICAL CONSTITUENTS AND LIPASE INHIBITORY ACTIVITY OF *Solanum stramonifolium* Jacq. FRUIT
By	Miss Watinee Chanmee
Field of Study	Chemistry
Thesis Advisor	Associate Professor Amorn Petsom, Ph.D.
Thesis Co-Advisor	Chanya Chaicharoenpong, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

S. Hannongbua ..... Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

Sirirat kokpol ..... Chairman

(Associate Professor Sirirat Kokpol, Ph.D.)

..... Thesis Advisor

(Associate Professor Amorn Petsom, Ph.D.)

Chanya Chaicharoenpong ..... Thesis Co-Advisor

(Chanya Chaicharoenpong, Ph.D.)

N. Ngamrojanavanich ..... Examiner

(Associate Professor Nattaya Ngamrojanavanich, Ph.D.)

Damrong Sommit ..... External Examiner

(Damrong Sommit, Ph.D.)

วาทินี จันมี : องค์ประกอบทางเคมีและฤทธิ์ยับยั้งไลเพสของผลมะอึก *Solanum stramonifolium* Jacq. (CHEMICAL CONSTITUENTS AND LIPASE INHIBITORY ACTIVITY OF *Solanum stramonifolium* Jacq. FRUIT) อาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.อมร เพชรสม, อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม : อาจารย์ ดร.จรรยา ชัยเจริญพงศ์, 78 หน้า.

ในงานวิจัยนี้ได้ทำการศึกษาองค์ประกอบทางเคมีและฤทธิ์ยับยั้งไลเพสของผลมะอึก (*Solanum stramonifolium* Jacq.) โดยการสกัดผลมะอึกแห้งด้วยเฮกเซน เอทิลอะซิเตตและน้ำตามลำดับ ทำการทดสอบความสามารถในการยับยั้งไลเพส พบว่า ส่วนสกัดหยาบทั้งสามให้ค่าเปอร์เซ็นต์การยับยั้งไลเพสเท่ากับ 33.4 ± 2.7, 94.6 ± 8.3 และ -1.1 ± 1.5 เปอร์เซ็นต์ ตามลำดับ ที่ความเข้มข้น 1.25 ไมโครกรัมต่อมิลลิลิตร จากนั้นนำส่วนสกัดหยาบเฮกเซน และ เอทิลอะซิเตต มาทำการแยกและทำให้บริสุทธิ์ด้วยเทคนิคคอลัมน์โครมาโทกราฟี พบสารบริสุทธิ์ 2 สารที่สามารถออกฤทธิ์ยับยั้งไลเพสได้ดี คือคาร์เพสเตอรอล และ ลิโนเลอิกเอซิด มีค่า IC_{50}เท่ากับ 56.0 และ 43.6 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ ในขณะที่ออลิสแตตมีค่า IC_{50}เท่ากับ 0.0035 ไมโครกรัมต่อมิลลิลิตร สำหรับการหากลไกการยับยั้งไลเพส พบว่าคาร์เพสเตอรอลเป็นตัวยับยั้งไลเพสแบบแข่งขัน และ ลิโนเลอิกเอซิดเป็นตัวยับยั้งไลเพสแบบไม่แข่งขัน มีค่าความสามารถในการจับกับเอนไซม์เท่ากับ 2.175 และ 2.981 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ ที่ความเข้มข้น 12.5 ไมโครกรัมต่อมิลลิลิตร ส่วนของการทดสอบประสิทธิภาพการทำงานของออลิสแตตร่วมกับคาร์เพสเตอรอล และ ลิโนเลอิกเอซิด พบว่าเป็นแบบต้านฤทธิ์กัน (antagonistic interactions)

ภาควิชา เคมี ลายมือชื่อนิสิต วาทินี จันมี

สาขาวิชา เคมี ลายมือชื่อ.ที่ปรึกษาวิทยานิพนธ์หลัก

ปีการศึกษา 2552 ลายมือชื่อ.ที่ปรึกษาวิทยานิพนธ์ร่วม จรรยา ชัยเจริญพงศ์

5072596023 : MAJOR CHEMISTRY

KEYWORDS : Lipase / *Solanum stramonifolium* Jacq. / Lipase inhibitor

WATINEE CHANMEE : CHEMICALCONSTITUENTS AND LIPASE INHIBITORY ACTIVITY OF *Solanum stramonifolium* Jacq. FRUIT. THESIS ADVISOR : ASSOC. PROF. AMORN PETSOM, Ph.D., THESIS CO-ADVISOR : CHANYA CHAICHAROENPONG, Ph.D., 78 pp.

The chemical constituents and lipase inhibitory activities from fruits of *Solanum stramonifolium* was studied. The fruits of *S. stramonifolium* were extracted by hexane, ethyl acetate and water, respectively. The lipase inhibitory activity of each crude was 33.4 ± 2.7, 94.6 ± 8.3 and -1.1 ± 1.5% inhibition at concentration 1.25 mg/mL, respectively. Therefore, the hexane and ethyl acetate crude extracts were selected and, then purified by using silica-gel column chromatography with hexane ethyl acetate gradient solvent system to give two compounds, carpesterol and linoleic acid, exhibited high inhibitory activity, showed IC_{50} 56.0 and 43.6 μg/ml, respectively while orlistat (positive control) showed IC_{50} 0.0035 μg/ml. For investigation of inhibitory properties of the active compounds was found that carpesterol is a competitive inhibitor and linoleic acid is a noncompetitive inhibitor of porcine pancreatic lipase with the K_i values of 2.175 and 2.981 μg/ml at concentration 12.5 μg/ml, respectively under the same condition. In terms of efficacy of combination of orlistat with carpesterol and linoleic acid are antagonistic interactions.

Department : Chemistry Student's Signature : Watinee Chanmee

Field of Study : Chemistry Advisor's Signature :

Academic Year : 2009 Co-Advisor's Signature : Chanya Chaicharoenpong

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and gratitude to Associate Professor Amorn Petsom, Ph.D., my advisor and Chanya Chaicharoenpong, Ph.D., co-advisor for their excellent suggestion, guidance, encouragement and supportive throughout the entire period of conducting this thesis.

I would also like to extend my gratitude to Associate Professor Sirirat Kokpol, Ph.D., Associate Professor Nattaya Ngamrojanavanich, Ph.D. and Damrong Sommit, Ph.D., for serving as the committee and for their editorial assistance and comments.

I would like to thank Associate Professor Chaiyo Chaichanthipyuth, Ph.D. for his kindness, suggestion and support.

I would like to thank the staffs of Institute of Biotechnology and Genetic Engineering, the staffs of Biotechnology Program and the staffs of Department of Chemistry, Faculty of Science, Chulalongkorn University for their support and help. The financial support Center form Petroleum, Petrochemicals, and Advanced Materials and the Graduate School of Chulalongkorn University is also gratefully acknowledged.

Finally, my deepest gratitude is to my family for their support, understanding, and encouragement throughout my study.

CONTENTS

CHAPTER Page

Page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

°C	Degree Celsius
µg	Microgram
µl	Microlitre
A	Absorbance
APGPR	(Ala-Pro-Gly-Pro-Arg) is a pentapeptide released from procolipase during fat digestion
ASTM	American Society for Testing and Materials
BMI	Body mass index
cDNA	Complementary DNA
cm	Centimeter
COSY	Correlation spectroscopy
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethyl sulfoxide
eV	Electron volt
g	Gram
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
kg	Kilogram
K_m	Michaelis-Menten constant
L	Litre
M	Molar
mg	Milligram
MHz	Megahertz
min	Minute
ml	Millilitre
mM	Millimolar
MS	Mass spectrometry
MW	Molecular weight

ng	Nanogram
NMR	Nuclear Magnetic Resonance
PNP	*p*-Nitrophenylplamitate
TLC	Thin layer chromatography
TOF	Time of flight
Tris	Tris(hydroxymethyl)-aminomethane
UV/VIS	Ultraviolet/visible
v/v	Volume by volume
V_0	Initial velocity
V_{max}	Maximum velocity
w/v	Weight by volume
w/w	Weight by weight

CHAPTER I

INTRODUCTION

Although widely regarded as a problem confined to the developed world, the obesity epidemic is, in reality, sweeping inevitably through the developing nations as well [1]. Obesity or hyperlipidemia develops, leading to a variety of serious diseases, including atherosclerosis, hypertension, diabetes and functional depression of certain organs. One of the most important strategies in the treatment of obesity includes development of inhibitors of nutrient digestion and absorption, in an attempt to reduce energy intake through gastrointestinal mechanisms, without altering any central mechanisms [2]. Since dietary lipids represent the major source of unwanted calories, specifically inhibiting triglyceride digestion forms a new approach for the reduction of fat absorption [3]. Orlistat, a hydrogenated derivative of lipstatin derived from *Streptomyces toxitricini*, is a potent inhibitor of gastric, pancreatic, and carboxyl ester lipases and has proved to be effective for the treatment of human obesity.

The existence of lipase inhibitors in various plant species has been investigated and reported in different species including such as *Cassia mimosoides* [4], *Camelia sinensis* [5] and *Salaci reticulate* [6]. Twenty methanol and water crude extracts from Thai-traditional medical plants [7] were screened for their ability to inhibit lipase activity. It was found that the methanol crude extract of fruit of *Solanum stramonifolium* Jacq. exhibited the highest inhibitory activity with IC_{50} value of 0.11 mg/mL.

S. stramonifolium Jacq. (hairyfruited pea-eggplant or Ma Euk) is a plant in Solanaceae family, of the Solanum genera, that diversified hill fields and agroforest orchards of Thailand and it is an economically important species for having multiplicity uses. The fruits are normally eaten raw at mealtimes.

Figure 1.1 *Solanum stramonifolium* Jacq. a: leave b: flower c: raw fruit d: ripe fruit

Objectives

To isolate and characterize chemical constituents with lipase inhibitory activity from *S. stramonifolium* fruit.

Scope of work

In initial work, fruits of *S. stramonifolium* were extracted with hexane, ethyl acetate and water then separated by chromatography technique. Afterwards, purified lipase inhibitors were identified by spectroscopic techniques and investigated theirs lipase inhibitory activity.

CHAPTER II

THEORETICAL

2.1 Lipase

Lipases are enzymes that digest fat. It includes phospholipases and triacylglycerol lipase. Phospholipase catalyzes the hydrolysis of phospholipids to free fatty acid and lysophospholipid. Triacylglycerol lipase catalyzes the hydrolysis of triacylglycerol to free fatty acid, mono- and diacylglycerols. Free fatty acids are employed for energy production or are re-esterified for storage in the adipose tissue [8 and 9]. The human lipases distinguish two parts. First, the pre-duodenal part includes lingual and gastric lipase. Second, the extra-duodenal part includes pancreatic, hepatic, lipoprotein and endothelial lipases that has a considerable molecular homology with lipoprotein lipase and hepatic lipase is synthesized by endothelial cell [10 - 11]. Basic review of human digestive and metabolic lipases is shown in Figure 2.1

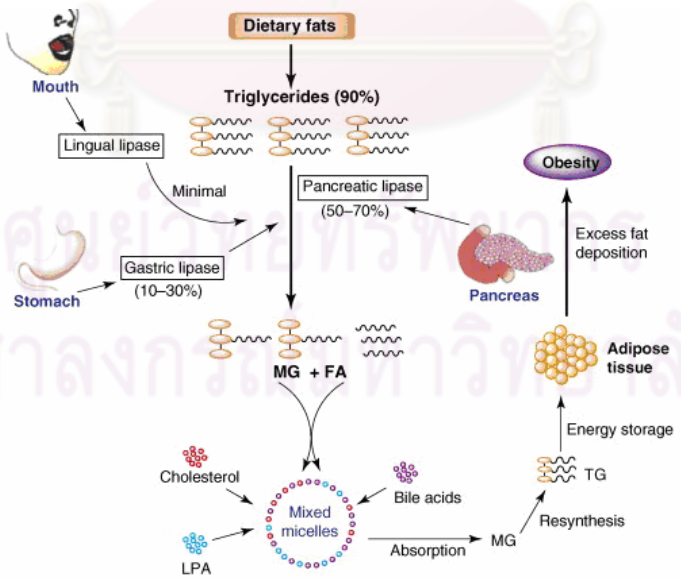


Figure 2.1 Physiological role of pancreatic lipase in lipid absorption. [12]

The predominant pre-duodenal lipase in humans; gastric lipase and lingual lipase is present in trace amounts. Gastric lipase is generated in the gastric juice by the chief cells of fundic mucosa in the stomach. In the saliva lingual lipase is generated by the serous von Ebner glands of the tongue secrete [13 - 14]. The gene encoding human gastric lipase has been localized on chromosome 10q23.2 [15 - 17]. The lingual and gastric lipases have greater pH stability and lower molecular weights than enzymes of the lipase superfamily.

The chromosomal localization of the genes encoding the extra-duodenal lipases and their tissue of origin have been described (Table 2.1). These enzymes share a high degree of primary sequence homology [18] and similar tertiary structure as suggested by mostly conserved disulfide bonds [19].

Table 2.1 Extra-duodenal lipase gene family

Lipase	Chromosomal localization of gene	Tissue of origin
Pancreatic lipase	10q26.1	Pancreas
Hepatic lipase	15q21–q23	Liver
Lipoprotein lipase	8p22	Adipose, heart, skeletal muscle
Endothelial lipase	18q21.1	Endothelial cells, liver, lung, kidney, placenta

2.1.1 Pancreatic lipase

Pancreatic lipase, the lipolytic enzyme is synthesized and secreted by the pancreas. It digests triglycerides with hydrolysis of 50–70% of total dietary fats, and it removes fatty acids from the α and α′ position of dietary triglycerides, yielding β-monoglycerides and the lipolytic products (long chain saturated and polyunsaturated fatty acids) [20-22]. This mechanism is shown in Figure 2.2. Moreover, the physiological role of pancreatic lipase, the three-dimensional structure of human pancreatic lipase was distinguished by X-ray crystallography. The primary structure was established by analysis of cDNA clones isolated from a human pancreas cDNA library and found to be a single chain glycoprotein of 449 amino acids. The encoded protein shows 86% and 68% homology with porcine and canine pancreatic lipase, respectively [23]. The polypeptide chain is divided into two folding units, the larger N-terminal domain, the catalytic domain, comprising amino acid residues 1-336 and a C-terminal domain, binding the colipase and containing amino acid residues 337–449 typical of β-sandwich type [24]. Domains required for activity in the structure of human pancreatic lipase are His-263, Asp-176 and Ser-152 form a triad, analogous to the serine proteases, called the lipolytic site. Enzymatic activity has shown to be decreased after chemical modification of Ser-152, located in the larger N-terminal domain at the C-terminal edge of a doubly-wound parallel β-sheet and is a part of Asp-His-Ser triad, thus indicating that Ser-152 is essential for the catalytic activity [23]. The determined X-ray crystal structures of lipases revealed. The reaction mechanism of lipases involved the lid covering the active site and the active site.

The lid of lipase usually contains a small α-helix or loop, which covers the active site pocket. These structures elucidate the enabled structural basis for the interfacial activation of lipases. The adsorption of lipase at the interface leads to restructuring of the enzyme and opening of the catalytic lid toward the hydrophobic phase. Therefore, this lid opened in the presence of lipid or organic solvent exposes the active site [25 - 26]. The lid is shown in Figure 2.3.

The active site, a pocket contains residues responsible for the substrate specificity and catalytic residues with esterification of triglyceride. First, An acyl

enzyme binds to a substrate with acylation, and deacylation provides alcohol and carboxylic acid that shown in Figure 2.4.

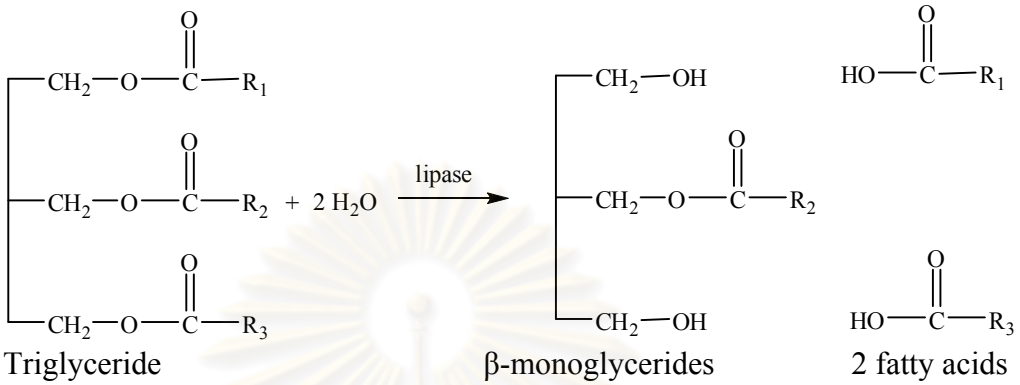


Figure 2.2 Hydrolysis of triglyceride by lipase.

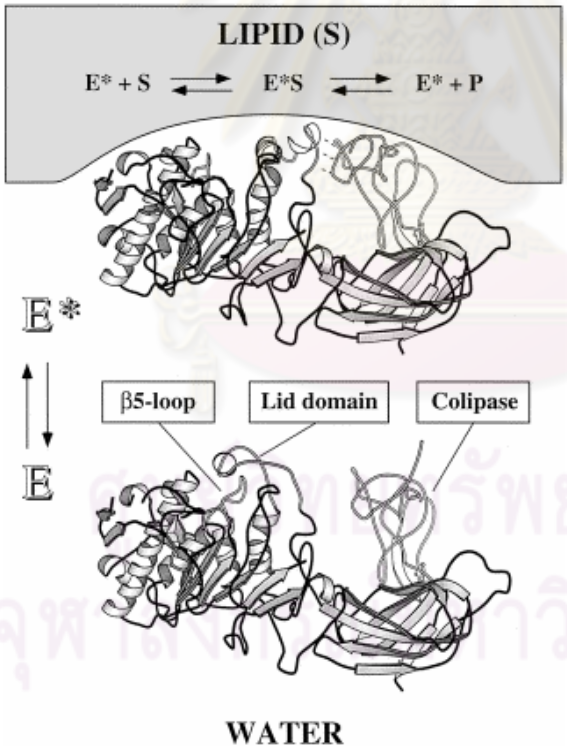


Figure2.3 Structure of human pancreatic lipase and conformational change induced by the adsorption to a lipid interface [26]

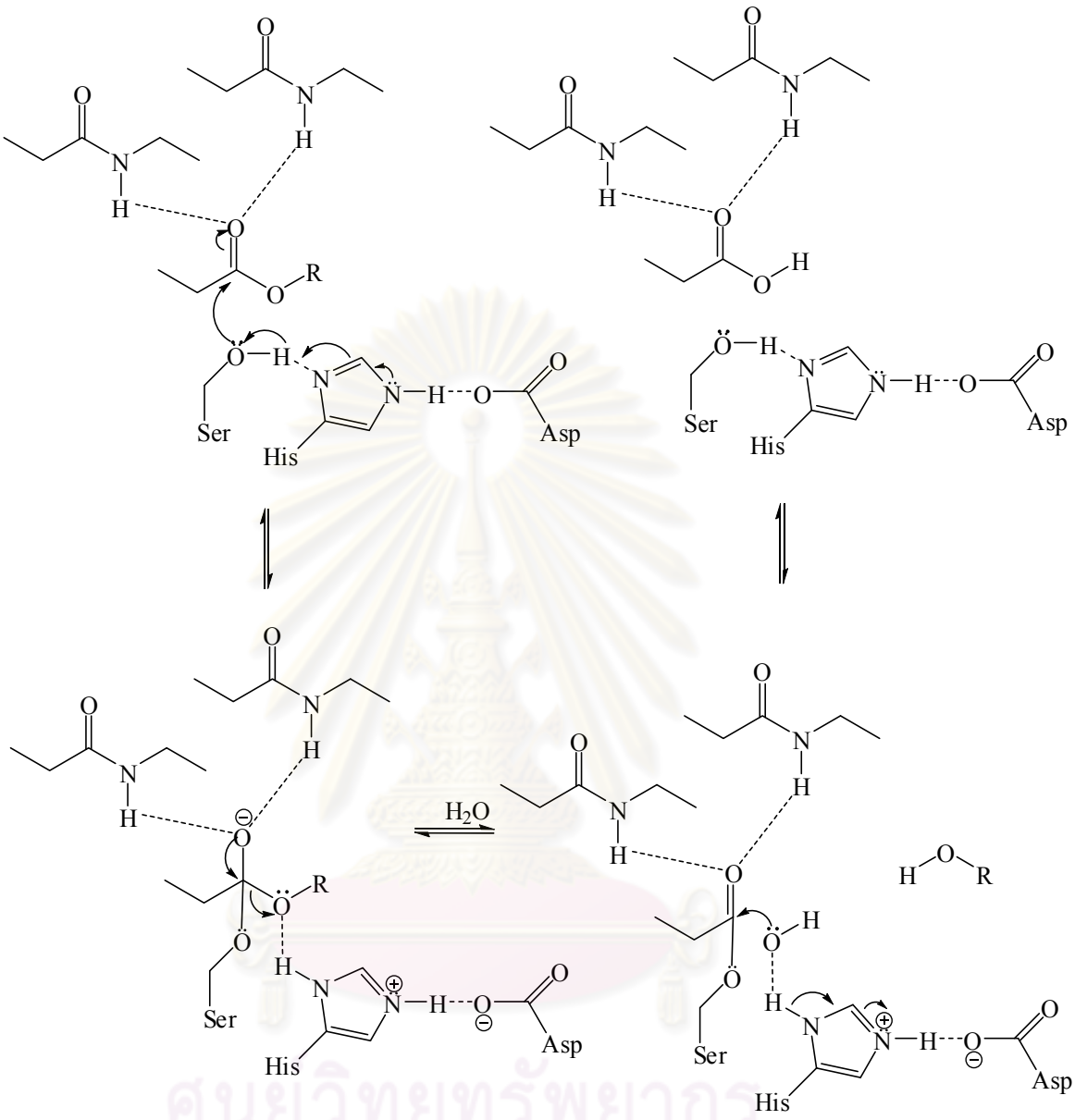


Figure 2.4 Hydrolysis of a butyrate ester catalyzed by lipase involves an acyl enzyme and two different tetrahedral intermediate.

Pancreas requires another pancreatic departure protein; colipase, for full activity. Colipase is secreted by the exocrine pancreas as a precursor molecule, procolipase, which is processed to mature colipase by cleavage of the procolipase propeptide (APGPR). Procolipase binds specifically to the C-terminal domain of the pancreas molecule, without inducing any conformational change [24]. Moreover, essential lipid hydrolases are lingual lipase and gastric lipase. Lingual lipase is

secreted by serous gland and digests approximately one third of ingested fat. Gastric lipase is secreted in response to mechanical stimulation, ingestion of food or sympathetic activation, accounts for the hydrolysis of 10–40% of dietary fat. Thus, potentially impact of the inhibition of lipid absorption that could result from the activity of only pancreatic lipase reduced [22].

2.2. Obesity

Obesity [27] is defined as a body mass index (BMI) of 30 or more, where BMI is a statistical measurement which compares a person's weight and height produce a unit of measure of kg/m^2. Overweight or obese is defined as a BMI between 25 and 29.9. (BMI cut-off values are ethnic-dependent and appear to be lower in certain populations: a BMI of 27.5 or greater in an Asian patient is associated with comparable morbidities to those seen in a Caucasian patient with a BMI of 30.) BMI can also be determined using a BMI chart shown in Figure 2.5

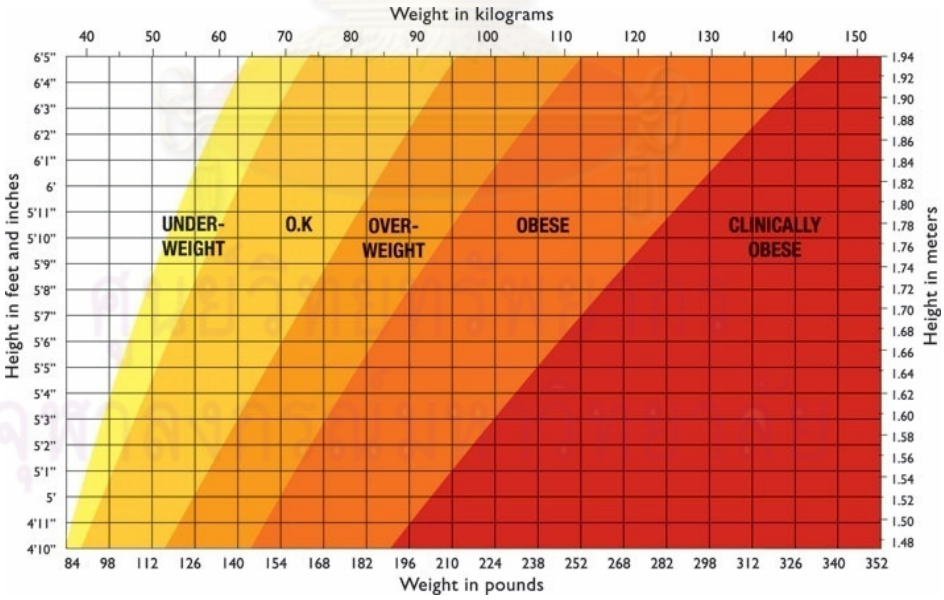


Figure 2.5 A graph of body mass index. Based on World Health Organization data

Obesity is diseases in which an excess of body fat has accumulated. It can cause various diseases such as cardiovascular diseases, diabetes mellitus type 2,

obstructive sleep apnea, certain types of cancer, and osteoarthritis. The causes of obesity are combination of excessive food energy intake and a lack of physical activity. A limited number of cases are due primarily to genetics, medical reasons, or psychiatric illness. Mechanisms of anti-obesity drug are directed at reducing energy food intake either by an action on the gastrointestinal system or reduce appetite and feeding via an action through the central nervous system control.

2.3 Anti-obesity drug

There are currently two categories of anti-obesity drugs. First type acts on the central nervous system to primarily suppress appetite, other type acts on the gastrointestinal system (pancreatic lipase inhibitors).

Drugs acting central nervous system: sibutramine serotonin and norepinephrine promote a sense of satiety through its central action. In addition, it may decrease against the fall in thermogenesis through stimulation of peripheral norepinephrine receptors.

Drugs acting on the gastrointestinal system: pancreatic lipase inhibitors: orlistat inhibits pancreatic and gastric lipase by decreasing among triglyceride which is hydrolyzed from fat taken. It produces a dose-dependent reduction in dietary fat absorption leading to weight loss in obese subjects.

The mushrooming market of anti-obesity drugs will not slow down within the foreseeable future [12]. Table 2.2 summarizes those anti-obesity drugs that are currently approved and investigational drugs.

Table 2.2 Current and investigational anti-obesity drugs

Drug	Target/mechanism
Current anti-obesity drugs on the market	
Orlistat	Pancreatic lipase
Sibutramine	Serotonin and noradrenaline reuptake inhibitor
Rimonabant	CB1 cannabinoid receptor antagonist
Selected antiobesity drugs in clinical trials	
ATL-962 (cetilistat)	Pancreatic lipase
GT389-255	Lipase inhibitor
APD356	Selective 5HT2C agonist
SLV319	*CB1 receptor Antagonist
CP945 598	CB1 receptor Antagonist
SR58611A	β 3-adrenergic receptor agonist
L796568	β 3-adrenergic receptor agonist
Metreleptin	Modified leptin
Leptin	Leptin receptor
GI181771	CCK-A agonist
Oleoyl estrone	
PYY(3–36)	Synthetic form of the appetite suppressing hormone PYY(3–36)
TM30338	Neuropeptide Y2 and Y4 agonists
Pramlintide	Delays gastric emptying
1426	Peripheral mechanism
CYT-009-GhrQb	Ghrelin-targeted vaccine
AOD9604	Human growth harmone
P57	Apetite suppressant

*Cannabinoid receptor type 1

Dietary lipids represent the major source of unwanted calories, specifically triglyceride. Development of inhibitors of nutrient digestion and absorption without altering any central mechanisms [21, 31 - 32] are one of the most important methods in the treatment of obesity, these are an attempt to reduce energy intake through gastrointestinal mechanisms.

Orlistat or tetrahydrolipstatin, lipase inhibitor obesity-drug, is the best-selling anti-obesity drugs worldwide that depend on knowledge about lipase inhibition from natural products. It is a chemically synthesized derivative of lipstatin, which is a natural product of *Streptomyces toxytricini* [33]. It is irreversible inhibitor of gastric and pancreatic lipases, and has no effect on other hydrolases .The drug reduces triglyceride hydrolysis and fat absorption by 30% [34]. Although, it has some seamy gastrointestinal side effects as oily spotting, oily stools and flatulence [35]. Mechanism of action and metabolism of orlistat that shown in figure 2.5 : the covalent bond between orlistat and lipase occur within the active serine of gastric and pancreatic lipases in the lumen of the gastrointestinal tract, and forms a stable complex [13]. The complex induces a conformational change in the enzyme that leads to a lid-like structure on the lipase, hence exposing the catalytic active site [18]. This operation leads to acylation of a hydroxyl group on serine residue burden on the activesite of the enzyme making it inactive as lipase. Two metabolites are excreted via the bile have been detected, namely: M1 and M3. They found to be with no pharmacological activity in comparison to parent compound. The half-life of M1 and M3 are approximately 2 hr and 3 hr, respectively. M1 result from the opening of β-lactone moiety in orlistat while the M3 metabolite as a result of both the hydrolysis of β-lactone and ester group at the N-formyl leucine side chain [25]. The drug prevents the hydrolysis of dietary fat into absorbable monoglyceride and free fatty acid [36]. In orlistat-treated patients, levels of both cholesterol and low-density lipoprotein are markedly reduced, which supports a role for triglyceride hydrolysis in cholesterol absorption [37].

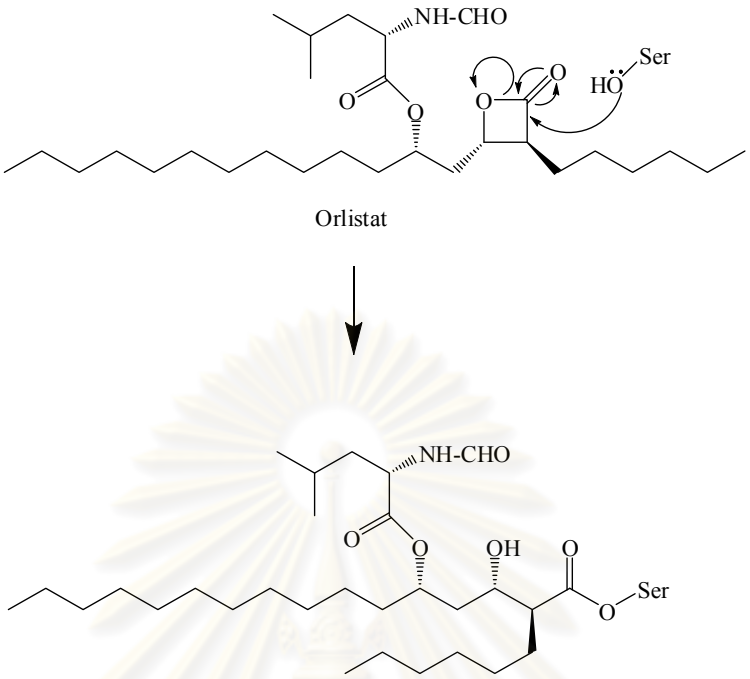


Figure 2.6 Mechanism on lipase inhibition of orlistat

At present, the potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective anti-obesity drugs. Over the counter remedies for obesity, based on nutritional supplements, are extremely popular; however, although such treatments are widely used, none has yet been convincingly demonstrated to be safe and effective.

2.4 *Solanum stramonifolium* Jacq.

S. stramonifolium (hairyfruited pea-eggplant or Ma Euk) is a plant in Solanaceae family, of the Solanum genera, that diversified hill fields and agroforest orchards of Thailand and it is an economically important species for having multiplicity uses. Ma Euk is hairy spherical shaped fruit which looks like tomato. The fruits are edible and herb. They are normally eaten raw at mealtimes and as anti-anemic and against tuberculosis [38 - 39].

2.5 Literature review

In 2006, twenty methanol and water crude extracts from Thai-traditional medical plants; the fruit of *Phyllanthus emblica* Linn., root of *Curcuma longa* Linn., fruit of *Solanum indicum* Linn., fruit of *Garcinia schomburgkiana* Pierre., fruit of *Solanum stramonifolium* Jacq., leave of *Ocimum americanum* Linn., leave of *Ocimum tenuiflorum* Linn., fruit and leave of *Phyllanthus acidus* Skeels. and leave of *Anethum graveolens* Linn., were screened for their ability to inhibit lipase activity. It was found that the crude methanol extract of fruit of *Solanum stramonifolium* Jacq. exhibited the highest inhibitory activity, IC_{50} value of 0.11 mg/mL [7].

Pancreatic lipase inhibitors from plants and microbial sources

Many metabolic products from microorganisms (bacterial, fungal and marine species) and phytochemicals identified from traditional medicinal plants have potent pancreatic lipase inhibitory activity. The review classifies these inhibitors into following chemical classes [12].

Table 2.3 Classification of Pancreatic lipase inhibitors from natural sources

Type	Chemical classes	Reference	Natural source	Inhibitor	IC_{50}
Saponins	Platycodin saponins	[40-44]	fresh roots of *Platycodin grandiflorum*	platycodin D **(1)**	-
	Scabiosaponins	[45]	*Scabiosa tschiliensis*	scabiosaponin E **(2)**	
				scabiosaponin F **(3)**	
				scabiosaponin G **(4)**	
				scabiosaponin I **(5)**	
				hookeroside A **(6)**	
				hookeroside B **(7)**	
				prosapogenin 1b **(8)**	
	Sessiloside and chiisanoside	[46]	leaves of *Acanthopanax sessiliflorusps*	sessiloside **(9)**	0.36 mg/mL
				chiisanoside **(10)**	0.75 mg/mL
	Chikusetsusaponins	[47]	rhizomes of *Panax japonicus* (ginseng roots)	Chikusetsusaponin III **(11)**	active at concentrations of 125–500 mg/mL
				chikusetsusaponin IV **(12)**	
				28-deglucosyl-chikusetsusaponins IV **(13)**	
				28-deglucosyl-chikusetsusaponins V **(14)**	
	Dioscin and derivatives	[48]	methanol extract of *Dioscorea nipponica*	dioscin **(15)**	20 µg/mL
				diosgenin **(16)**	28 µg/mL
				gracillin **(17)**	28.9 µg/mL
	Escins	[49]	Japanese horsechestnut (*Aesculus turbinate*) and European horsechestnut seeds (*Aesculus hippocastanum)*	escins Ib **(18)**	24 µg/mL
				escins IIb **(19)**	14 µg/mL
				escins Ia **(20)**	48 µg/mL
				escins IIa **(21)**	61 µg/mL
	Teasaponins	[50 - 51]	oolong, green and black tea	Teasaponins (a mixture of theasaponins *E1* and *E2*)	-
	Cyclocariosides	[52]	leaves of *Cyclocarea paliurus.*	cyclocarioside A **(22)**	9.1 mg/L
				cyclocarioside II **(23)**	-
				cyclocarioside III **(24)**	-

Table 2.3 (cont.) Classification of pancreatic lipase inhibitors from natural sources

Type	Chemical classes	Reference	Natural source	Inhibitor	IC_{50}
Polyphenolics	Polyphenolics	[53 - 54]	hot water extracts of various plant material	Luteolin **(25)**	weak inhibitor
		[55 - 56]	rhizomes of *Alpinia officinarum*	3-methyletherganglin **(26)**	moderate inhibition
			fruits of *Mangifera indica*	mangiferin **(27)**	moderate inhibition
			the peels of *Citrus unshiu*	Hesperidin **(28)**	32 μg/mL
	Oolong tea polyphenols	[57]	oolong tea	(−)-epigallocatechin 3,5-di-*O*- gallate **(29)**	0.098 μM
				prodelphinidin B-2 3,3′-di-*O*-gallate **(30)**	0.107 μM
				assamicain A **(31)**	0.120 μM
				oolonghomobisflavan A **(32)**	0.048 μM
				oolonghomobisflavan B **(33)**	0.108 μM
				theasinensin D**(34)**	0.098 μM
				oolongtheanin 3′-*O*-gallate **(35)**	0.068 μM
				theaflavin**(36)**	0.106 μM
				theaflavin 33′-*O*-gallate **(37)**	0.092 μM
Polyphenol rich extracts	Grape seed extract	[58]	Grape seed extract	proanthocyanidin-rich extracts	-
	Nelumbo nucifera extract	[59]	Blend tea or extract of leaf of *N. nucifera*	phenolic constituents of the leaves	0.46 mg/mL
	Salacia reticulata hot water extract	[60]	hot water extract of *S. reticulata* roots	a high concentration of polyphenols including mangiferin, catechins and condensed tannin	264 μg/mL
	Peanut shell extract	[61]	*Arachis hypogaea* or peanut shell extract	coumarin derivatives and flavonoid glycosides	-
	Mangifera indica leaf and stem bark extracts	[62]	*Mangifera indica* leaf and stem bark extracts	polyphenols, including phenolic acids, phenolic esters, flavan-3-ols and mangiferin	-
	CT-II extract	[63]	aqueous ethanol extract of *Cassia nomame* fruits	2S)-3′,4′,7-trihydroxyflavan-(4 α → 8)-catechin **(38)**	5.5 μM
Terpenes	Carnosic acid	[64]	methanol extract of *Salvia officinalis* leaves	carnosic acid **(39)**	12 μg/mL
				carnosol **(40)**	4.4 μg/mL
				roylenoic acid **(41)**	35 μg/mL
				7-methoxyrosmanol **(42)**	32 μg/mL
				triterpene oleanolic acid **(43)**	83 μg/mL
	Crocin and crocetin	[65 - 66]	*Gardenia jasminoids*	crocin **(44)**	28.63 μmol
				crocetin **(45)**	-

Table 2.3 (cont.) Classification of Pancreatic lipase inhibitors from natural sources

Type	Chemical classes	Ref.	Natural source	Inhibitor	IC_{50}
Pancreatic lipase inhibitors from microbial sources	Lipstatin	[67 - 68]	*Streptomyces toxytricini*	lipstatin **(46)**	0.14 μM
	Panclicins	[69 - 70]	*Streptomyces* sp. *NR 0619.*	panclicins A **(47)**	2.9μM
				panclicins B **(48)**	2.6μM
				panclicins C **(49)**	0.62μM
				panclicins D **(50)**	0.66μM
				panclicins E **(51)**	0.89 μM
	Valilactone	[71]	*Streptomyces albolongus strain MG147-CF2*	valilactone **(52)**	0.14 ng/mL
	Ebelactones	[72]	*Streptomyces aburaviensis strain G7-Gl*	ebelactone A **(53)**	3 ng/mL
				ebelactone B **(54)**	0.8 ng/mL
	Esterastin	[73]	*Streptomyces lavendulae* strain MD4-C1	esterastin **(55)**	0.2 ng/mL
	Caulerpenyne	[74]	*Caulerpa taxifolia*	caulerpenyne **(56)**	2 mM
	Vibralactone	[75]	*Boreostereum virans*	vibralactone **(57)**	0.4 μg/mL
	Percyquinin	[76 - 77]	*Basidiomycete stereum complicatum,* ST 001837	percyquinin **(58)**	2 μM

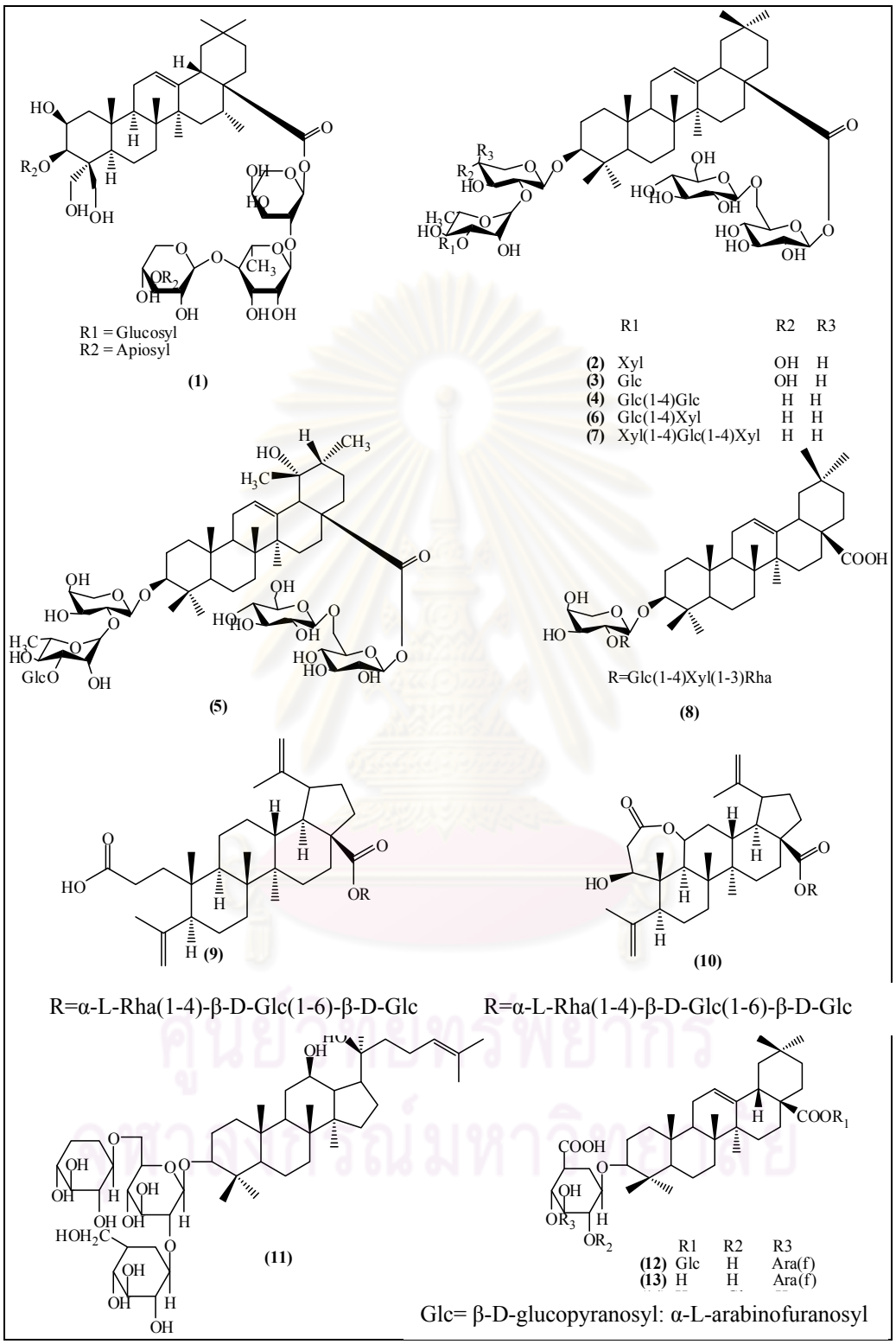


Figure 2.7 Structures of saponins with pancreatic lipase inhibitory activity.

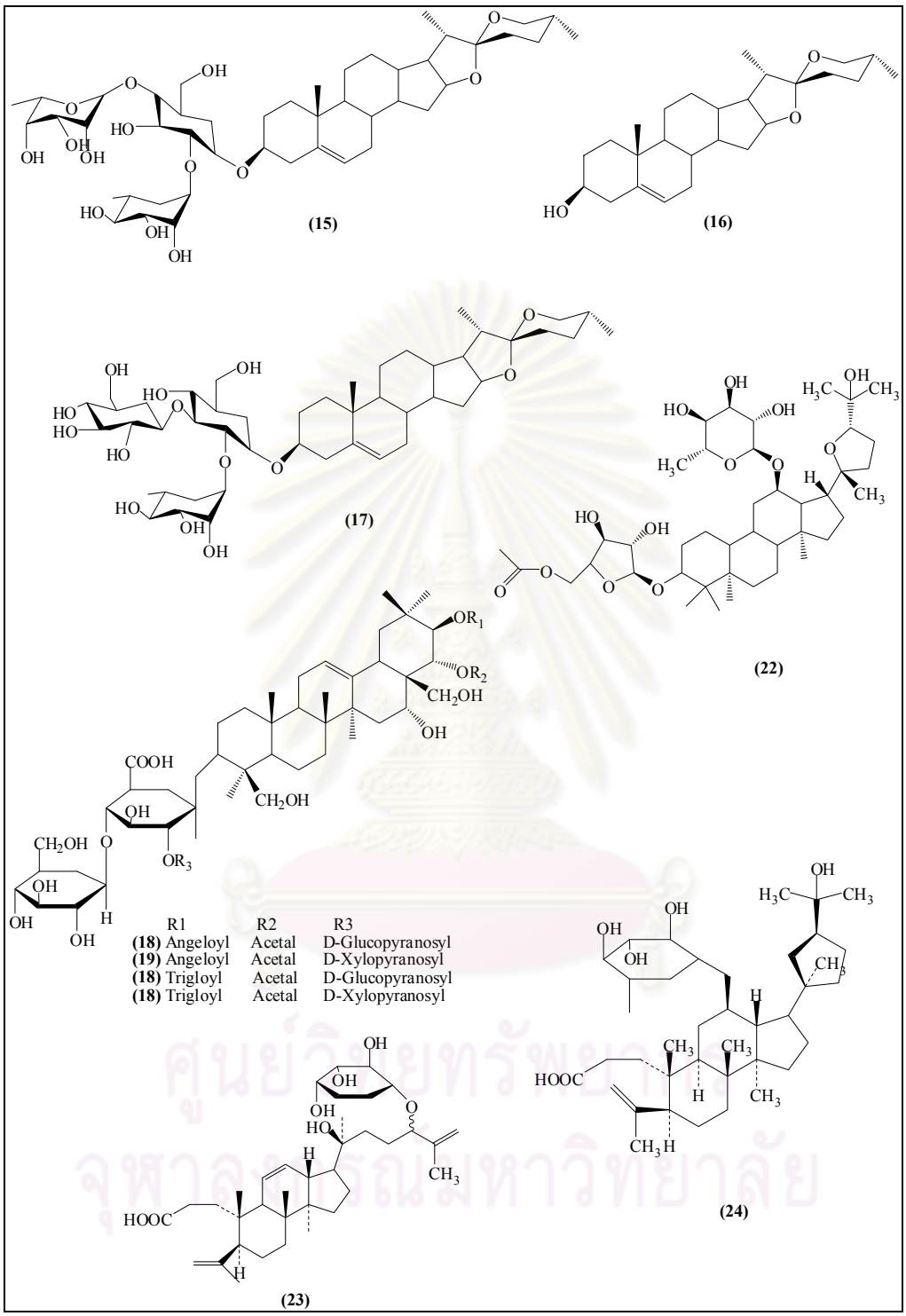


Figure 2.7 (cont.) Structures of saponins with pancreatic lipase inhibitory activity.

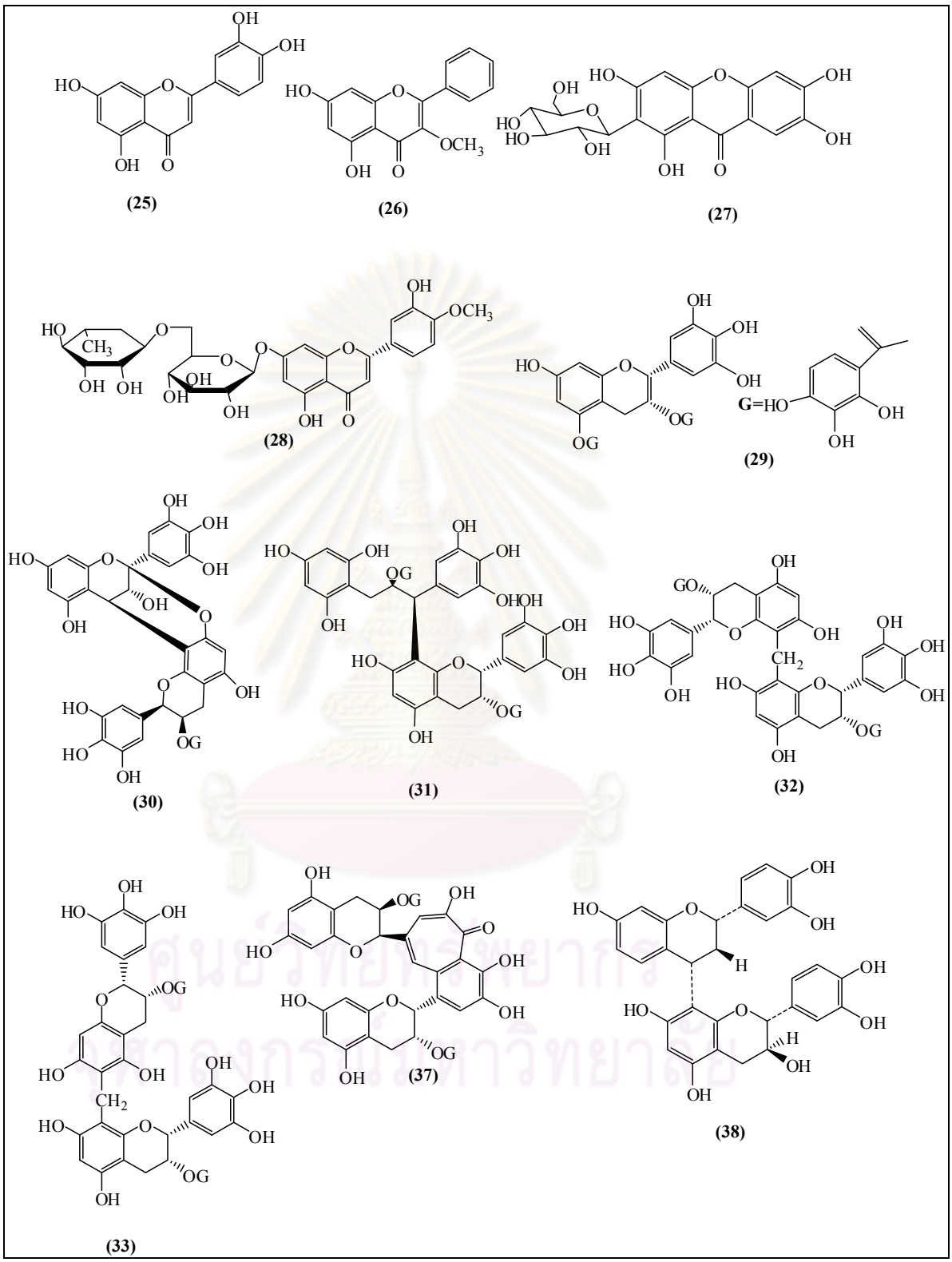


Figure 2.8 Structures of polyphenolic pancreatic lipase inhibitors.

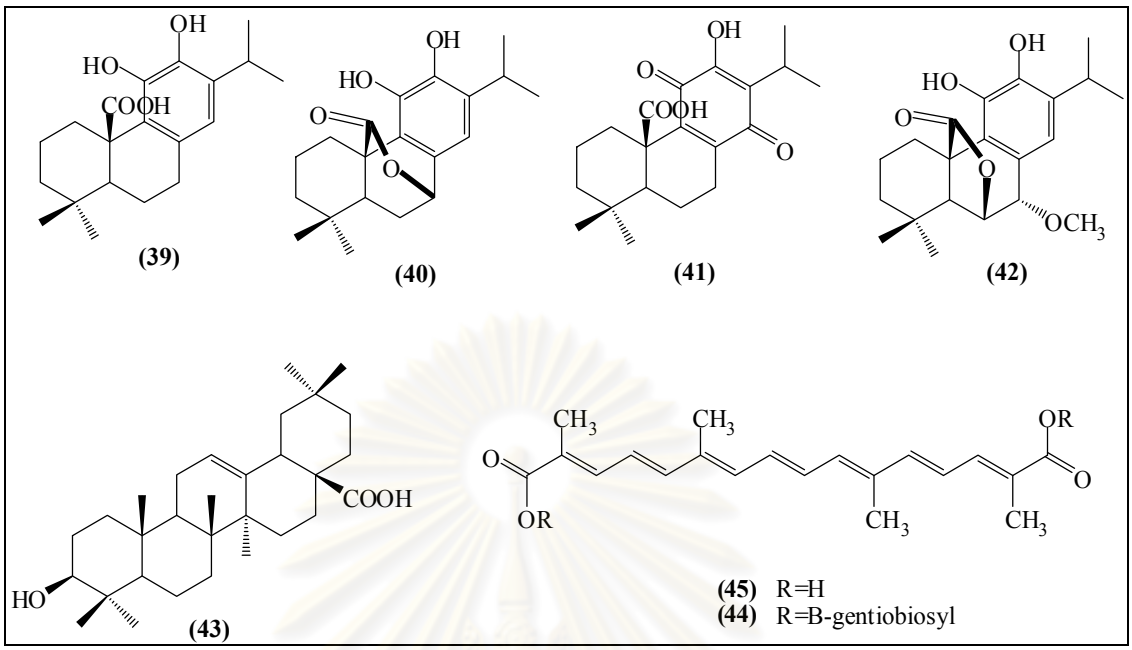


Figure 2.9 Structures of terpenoidal pancreatic lipase inhibitors.

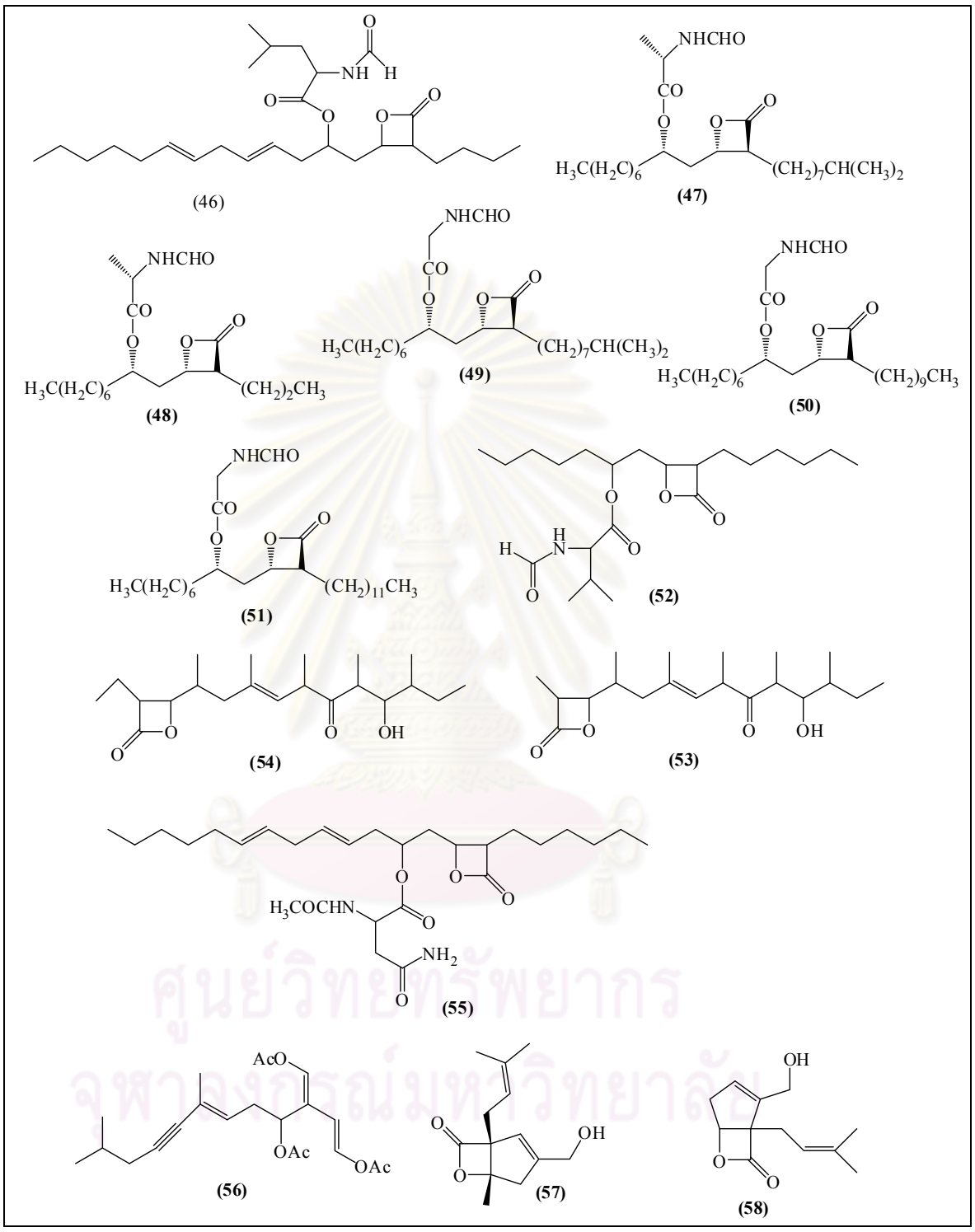


Figure 2.10 Structures of pancreatic lipase inhibitors from microbial sources

CHAPTER III

EXPERIMENTAL

3.1 Biological materials

Fruits of *S. stramonifolium* purchased from the Thonburi Railway market Bangkok those were collected in Amphoe Nakhon-chai-si, Nakhon-pathom province Thailand in 2008.

3.2 Instrument and equipments

3.2.1 Analytical thin layer chromatography (TLC)

Technique : One dimension, ascending

Absorbent : Silica gel 60 F_{254} precoated plate (E. Merck)

Layer thickness : 0.2 mm

Developing distance : 4.2 cm

Temperature : Laboratory room temperature (30-35 °C)

Detection : a. Ultraviolet light at wavelength of 254 and 365 nm

b. 10% H_2SO_4 in ethanol

3.2.2 Conventional column chromatography

Absorbent : Silica gel 60 (No. 7734) (E. Merck)

Particle size 0.063 – 0.200 nm. (70 – 230 mesh ASTM)

Packing method : Wet packing

Sample load : The sample was dissolved in small amount of eluent, and then mixed the sample with silica gel. Next, the mixture was dried and applied gently on top of the column.

Detection : Fractions were examined using TLC technique. In order to detect the compounds in each, the TLC plate was observed under UV light at wavelength of 254 and 365 nm and then exposed to 10% H_2SO_4 in ethanol and heating on a hot plate.

3.2.3 Spectroscopic techniques

3.2.3.1 UV/VIS spectrophotometer

UV/VIS spectra were obtained on a Synergy HT Muti – Detection Microplate Reader Bio-Tek Instrument at the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University.

3.2.3.2 Mass spectrometer (MS)

Time of flight spectra (TOF) of isolated compounds were obtained on a Micromass Platform II mass spectrometer at 70 eV at the Central Laboratory and Scientific Information Center for Halal Food Development, Chulalongkorn University.

MS scan : 50 – 1500 m/z in positive and negative mode

MS diluted solvent : 60% isopropanol, 28% methanol, 10% water, 1% formic acid and 1% ammonium hydroxide

Flow rate : 5 μL/min

3.2.3.3 Nuclear Magnetic Resonance Spectrometer (NMR)

The ^{1}H and ^{13}C nuclear magnetic resonance spectra of isolated compounds were recorded at 600.18 and 150.92 MHz, respectively, on a Bruker Model AC-F200 Spectrophotometer in deuterated chloroform. Chemical shift are given in parts per

million using residual protonated solvent as reference. HMQC, HMBC and COSY experiments were performed on the JEOL JNM-A500 Spectrometer

3.2.4 Rotatory evaporator under reduce pressure

The Eyela rotatory evaporator model N-1 was used for the rapid removal of large amounts of volatile solvents.

3.2.5 Melting point recorder

The melting point was recorded on Fisher-Johns melting point apparatus.

3.3 Chemicals and reagents

1. All commercial grade solvents used in this research; hexane, ethyl acetate and methanol were purified by distillation prior to use
2. Tris[hydroxymethyl]aminomethane (Tris-base), MB Grade, USB, USA
3. Hydrochloric acid, Merck, Germany
4. Dimethyl sulfoxide, ACS reagent, Fluka, Switzerland
5. Ethanol, ACS reagent, Riedel-de Haën, Germany
6. Acetonitrile, ACS reagent, Merck, Germany
7. *p*-Nitrophenylpalmitate, Sigma, Germany
8. Lipase from porcine pancreas Type II, 100-400 units/mg protein, Sigma, USA.

3.4 Extraction and isolation

3.4.1 Extraction

The fruits of *S. stramonifolium* were ground and dried, then soaked in hexane for three days at room temperature for two times. The liquid was centrifuged and then removed solvent by rotary evaporator to give hexane crude extract. After that

the discard was soaked in ethyl acetate for three days at room temperature for two times. The liquid was centrifuged and then removed solvent by rotary evaporator under reduce pressure to give ethyl acetate crude extract. Then the discard was extracted with boiled water and then removed water by rotary evaporator to give crude water extract. The procedure and results of the extraction are shown in Figure 3.1

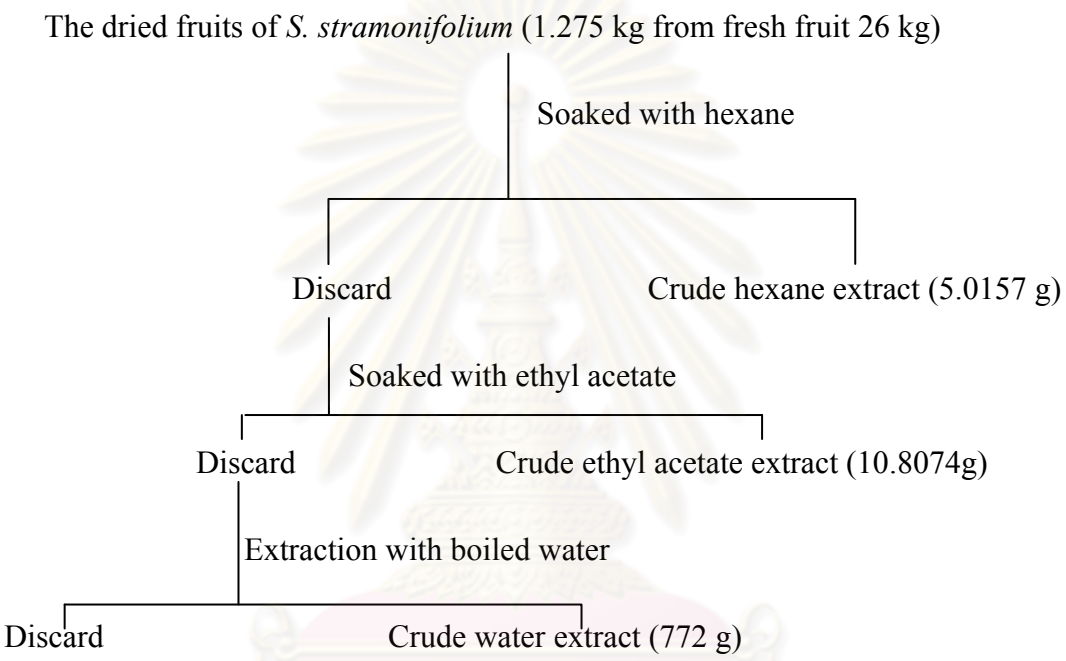


Figure 3.1. The procedure of extraction of *S. stramonifolium* fruits

Table 3.1 The crude extracts of *S. stramonifolium* fruit with various solvents

Solvent extract	Appearance	Wight (g)	% w/w of the dried plant
Hexane	Green-yellow oil	5.0157	0.40
Ethyl acetate	Dark- brown oil	10.8074	8.65
Water	Dark- brown gummy	772	60.55

3.4.2 Isolation of crude extracts of *S. stramonifolium* fruit

3.4.2.1 Separation of hexane crude extract

The hexane crude extract was obtained as green-yellow oil (5.0157 g, 0.40%). It was dissolved in hexane and filtered to give slightly green pellet crystal, then re-crystallized with hexane and ethyl acetate gave white pellet crystal of Compound 1.

3.4.2.2 Separation of ethyl acetate crude extract

The ethyl acetate crude extract was obtained as dark-brown oil (10.8074 g, 8.651%) after evaporation. It was fractionated by conventional column chromatography using Merck' silica gel 60, No. 7734 (70 – 230 mesh ASTM) as an adsorbent. The column was eluted with hexane-ethyl acetate gradient in stepwise fashion to give Compound 1, as white pellet crystal, and Compound 2 as slightly yellow wax.

3.5 Measurement of inhibitory pancreatic lipase activity *in vitro*

The method of Slanc *et a.l* [78] was employed with some modifications(stop the reaction with ethanol). *p*-Nitrophenylpalmitate (PNP) (Sigma) was dissolved in acetonitrile to give a stock solution with a concentration of 20 mM. Ethanol was then added to a final composition of acetonitrile:ethanol (1:2 v/v), resulting in 6.66 mM PNP. The solution was stored at -20 °C. The test sample was dissolved in water or DMSO (50 mg/mL). Porcine pancreatic lipase (type II, crude, Promega) was dissolved in 0.061 M Tris-HCl buffer (pH 8.5) to a final concentration of 5 mg/mL. 600 µL Reaction mixture, containing 0.60 mg/mL of enzyme(72 µL), 2.5 mg/mL of crude extracts (15 µL), 0.333 mM of PNP(30 µL) and 0.061 M of Tris-HCl buffer(483 µL), was incubated at 37 °C for 25 min. Then 600 µL ethanol was added to the mixture to stop the reaction. The absorbance of released *p*-nitrophenol was measured at 405 nm using a microplate reader. The blank was added ethanol before enzyme. The extract was replaced by water or DMSO for the control. The absorbance of samples were corrected by subtracting the absorbance of the blanks. Enzyme

activity was defined in terms of the increase of absorbance per minute. The difference between the enzyme activity of the control and the sample was defined as the inhibitory activity of the extract. DMSO, acetonitrile, ethanol and methanol at the concentrations used did not interfere with the reaction conditions. The activity assay was performed in triplicate for each crude extract, the results were averaged and expressed with standard deviations. A solution of orlistat was prepared by dissolving 120 mg of orlistat in DMSO as positive control. The A405 of liberated *p*-nitrophenol was measured with *p*-nitrophenol as a standard. One unit was defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol per minute under assay conditions.

$$\%\text{inhibition} = [(A-B)/A]x100$$

When "A" was the means of absorbance of control (without inhibitor and only DMSO at the same volumn being used for tested samples)

"B" was the means of absorbance of sample (with inhibitor)

3.6 Determination of the kinetic properties of pancreatic lipase

The pancreatic lipase was assayed using 3.996, 3.33, 1.665, 1.11 and 0.8325 mM of PNP. The Linewaver-Burk plot was created to analyze for K_m and V_{max} value using the following equation.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

When V_0 = initial velocity

V_{max} = maximum velocity

[S] = concentration of substrate

K_m = $V_{max}/2$ = Michaelis-Menten constant

3.7 Determination of protein of pancreatic lipase

Protein content of pancreatic lipase was determined by the method of Lowry *et a.l* [79]. Porcine pancreatic lipase (type II, crude, Promega) was dissolved in 0.061 M

Tris-HCl buffer (pH 8.5) to a final concentration of 5 mg/mL. 3 mL of alkaline copper reagent (0.01% w/v copper sulfate, 0.02% w/v sodium potassium tartrate and 2% w/v sodium carbonate in 0.1 N sodium hydroxide) was added to 0.1 mL of the lipase solution, mixed well and allowed to stand for 10 min. at room temperature. Then 0.3 mL of diluted Folin phenol reagent : deionized water (1:3) was added and incubated for 30 min. The absorbance of the mixture was measured at 650 nm using a microplate reader. The blank was deionized water. Protein content was calculate from a standard curve of Bovine Serum Albumin (BSA)

The standard curve plots obtained from the variation of BSA concentration : o.1, 0.2, 0.4, 0.60, 0.80, 1.0, 1.2, 1.6 and 2.0 mg/mL and deionized water was the blank.

3.8 Determination of IC_{50} value of isolated compounds

The IC_{50} value of isolated Compound 1 was determined by dissolved in DMSO (5.00, 1.00, 0.50, 0.25 and 0.10 mg/mL). Concentration of Compound 2 was prepared as 10.00, 1.00, 0.50, 0.10 and 0.005 mg/mL for biological assay. Then all prepared solutions were investigated for lipase inhibitory activity compared with orlistat, a positive control, (0.4, 0.05, 0.025, 0.0125 and 0.00625 mg/L) according to the procedure 3.5.

3.9 Investigation of inhibitory properties of isolated compound

The K_i value of the enzyme for the isolated compounds were determined by using 3.996, 3.33, 1.665, 1.11 and 0.8325 mM of PNP as substrate and the inhibitor concentrations of 3.00, 2.00 and 1.00 mg/mL for the isolated compounds were used. The Ki values were determine from the Lineweaver-Burk plot.

3.10 Determination of efficacy of combination of orlistat with isolated compounds

The efficacy of combination of oslistat with isolated compounds was determined by using combination of 50, 25, 10, 2.5 and 1.25 µg/L of orlistat with 6.25

μg/mL of Compound 1 for each concentration of orlistat. On the part of Compound 2 of 6.25 μg/mL combine with each concentration of orlistat for biological assay.

3.11 Statistical analysis

All data were presented as the mean ± SEM. Significant differences among the group were determined by *one-way t-test at p=0.05*.

CHAPTER IV

RESULTS AND DISCUSSION

The lipase inhibitor from fruits of *S. stramonifolium* was analyzed using all methods as described in the chapter III. The results have been shown and discussed in each part of this chapter, respectively.

4.1 Determination of the kinetic properties of pancreatic lipase

4.1.1 Determination of one lipase unit

Determination of absorbance at 405 nm of standard *p*-nitrophenol solution at 0.00 – 0.18 mM giving absorbance 0.000-1.473 U have been shown in Table 4.1. Then plotting standard curve were derived from this data in linear relationship that shown in Figure 4.1. Next, lipase 2.5 mg/mL were determined absorbance provide determination of one lipase unit 2.39 ± 0.08 μg (one unit is defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol per minute).

Table 4.1 Absorbance of standard *p*-nitrophenol solution at 405 nm

p-Nitrophenol (mM)	Absorbance (U)
0.00	0.000
0.03	0.285
0.06	0.527
0.09	0.765
0.12	0.993
0.15	1.250
0.18	1.473

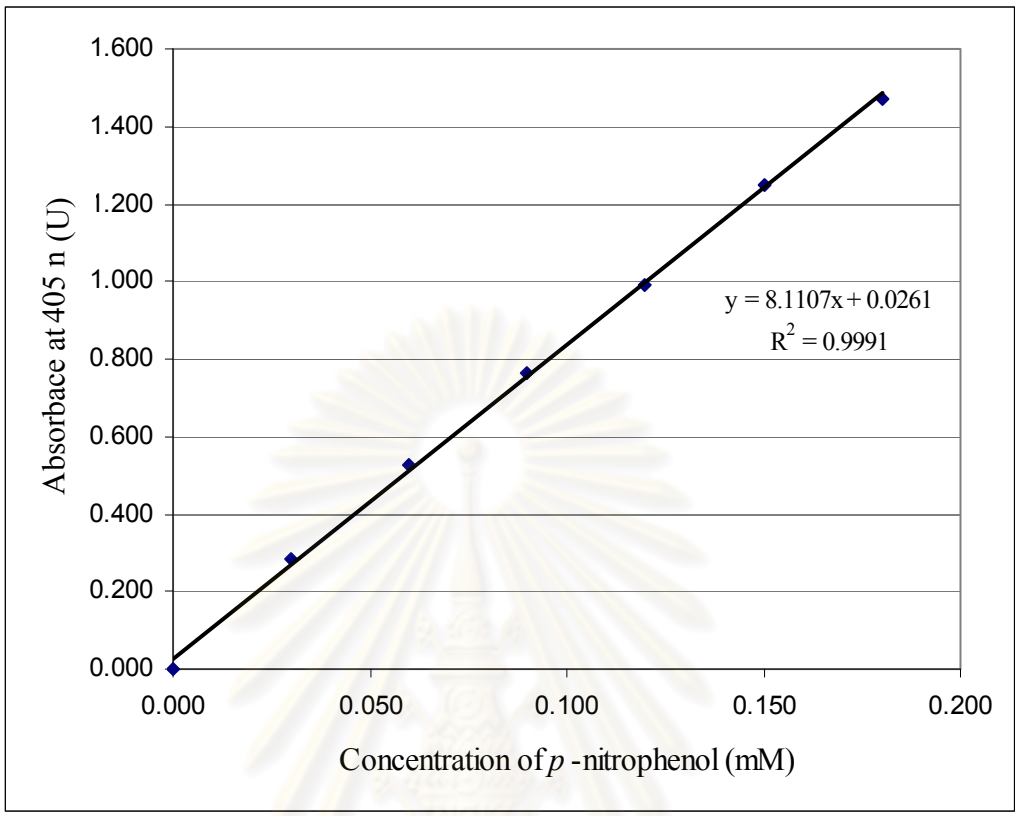


Figure 4.1 Standard curve of standard *p*-nitrophenol solution

Table 4.2 One lipase unit and absorbance of liberated *p*-nitrophenol

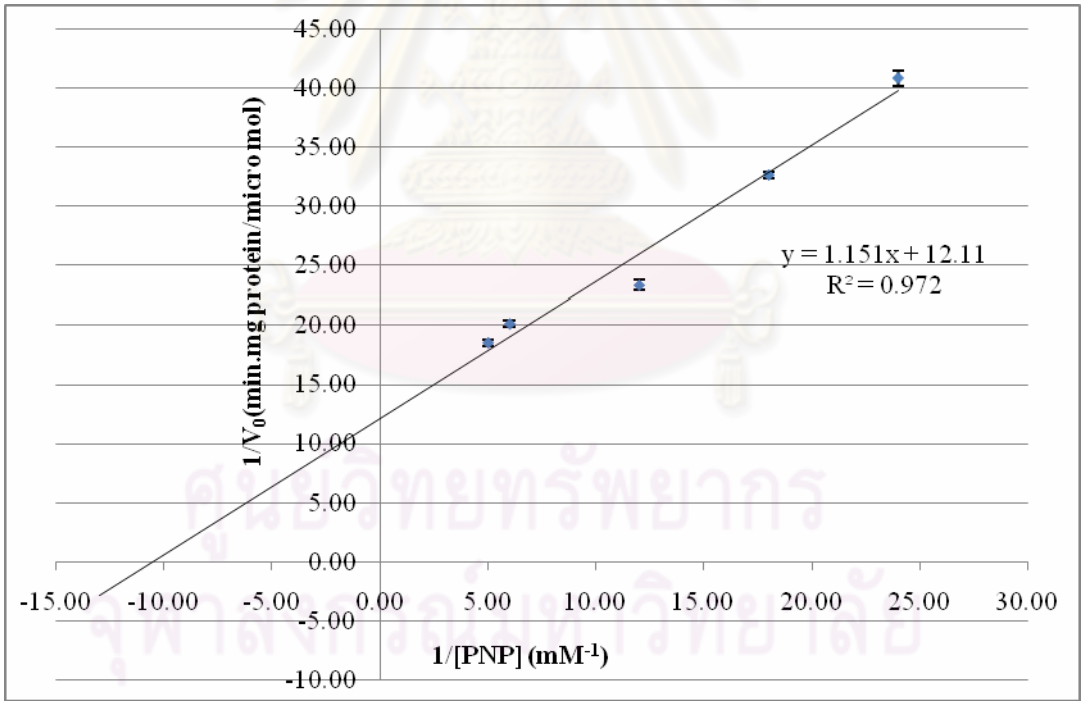
Absorbance(U)		One lipase unit* (μg)
I	0.543	2.36
II	0.518	2.48
III	0.550	2.33
Average	0.537	2.39 ± 0.08

* One unit (U) is defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol per minute.

4.1.2 Determination of K_m value of pancreatic lipase

Kinetic properties of lipase were shown in Figure 4.2. When concentration of PNP are increased, the initial velocities were increased. The initial velocities (V_0) were constant. The K_m and V_{max} values for the substrate of this enzyme were 0.095 mM^{-1} and 0.083 U/mg protein, respectively. The Michaelis constant K_m is experimentally defined as the concentration at which the rate of the enzyme reaction is half of V_{max} (the maximum reaction rate). K_m value includes the affinity of substrate for enzyme, but also the rate at which the substrate bound to the enzyme is converted to product.

Figure 4.2 Lineweaver-Burk plot analysis of the kinetic of lipase. The X-interception is $1/K_m$ and Y-interception is $1/V_{max}$



4.2 Inhibitory activity of crude extract on pancreatic llipase

In order to study and investigate the biological activity of the fruits of *S. stramonifolium,* the three crude extracts were at first tested for their inhibitory activities by using the modified method of Slanc *et al* [78]. The results were sumerized in Table 4.3.

Table 4.3 Percentage inhibition of crude extracts from the fruits of *S. stramonifolium*

Samples	Final Concentration (mg/mL)	%inhibition
Hexane crude extract	1.25	33.4 ± 2.7
Ethyl acetate crude extract	1.25	94.6 ± 8.3
Water crude extract	1.25	-1.1 ± 1.5
Orlistat	1×10^{-5}	102.5 ± 5.1
DMSO	-	0.0

From the data in Table 4.3, the water crude extract of fruits of *S. stramonifolium* did not show inhibitory activity on lipase as compare to positive control, orlistat. In spite of that, the hexane and ethyl acetate crude extracts exhibit more inhibitory activity with %inhibition of 33.4 ± 2.7% and 94.6 ± 8.3%, respectively than that of water crude extract, %inhibition of -1.1 ± 1.5%. Therefore, the hexane and ethyl acetate crude extract were further purified by silica-gel column chromatography with hexane ethyl acetate gradient solvent system to give two compounds; Compound 1, as white pellet crystal, and Compound 2 as slightly yellow wax.

4.3 lipase inhibitory activity of Compound 1 and 2

Table 4.4 Percentage inhibition of each isolated compounds

Sample	Concentration(µg/mL)	%inhibition
Compound 1	125	62.30 ± 6.19
	25	31.23 ± 6.16
	12.5	22.57 ± 5.75
	6.25	5.03 ± 10.64
	2.5	-3.67 ± 8.28
Compound 2	250	82.67 ± 0.45
	25	46.10 ± 3.14
	12.5	29.47 ± 0.75
	2.5	30.20 ± 3.73
	0.125	28.83 ± 3.23
Olistat	0.02	91.50 ± 0.56
	0.01	84.00 ± 1.48
	0.005	63.23 ± 5.77
	0.0025	33.07 ± 12.49
	0.0013	15.60 ± 6.66
DMSO	-	0

From the results in Table 4.4, the IC_{50} values of Compound 1 and 2 were determined by graph plotting between concentration of sample (x-axis) and % inhibition (y-axis). To determine IC_{50} values, a perpendicular line was drawn from the y-axis at the %inhibition value of 50 to the x-axis as shown in Figure 4.3 – 4.5. The IC_{50} values of orlistat and isolated compounds were summarized in Table 4.3.

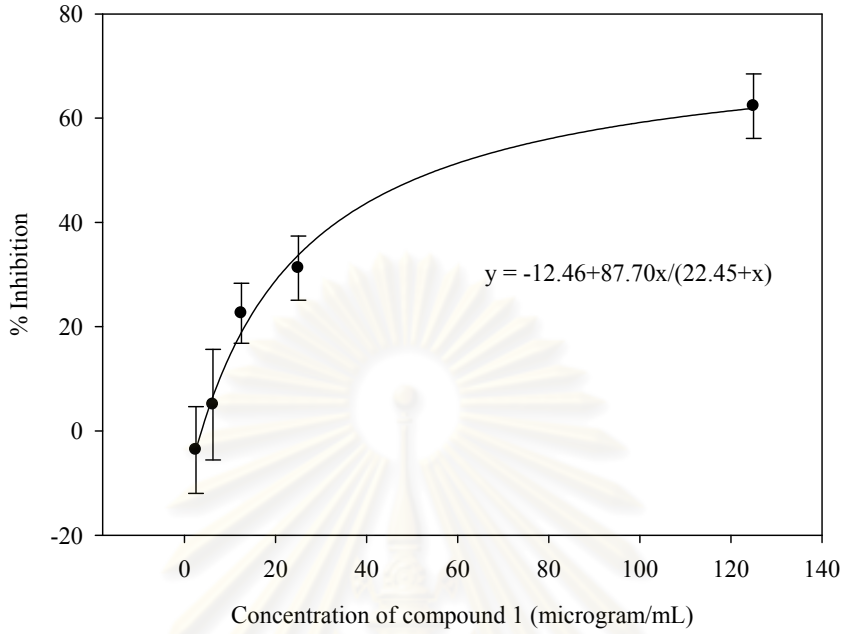


Figure 4.3 Effect of Compound 1 on pancreatic lipase

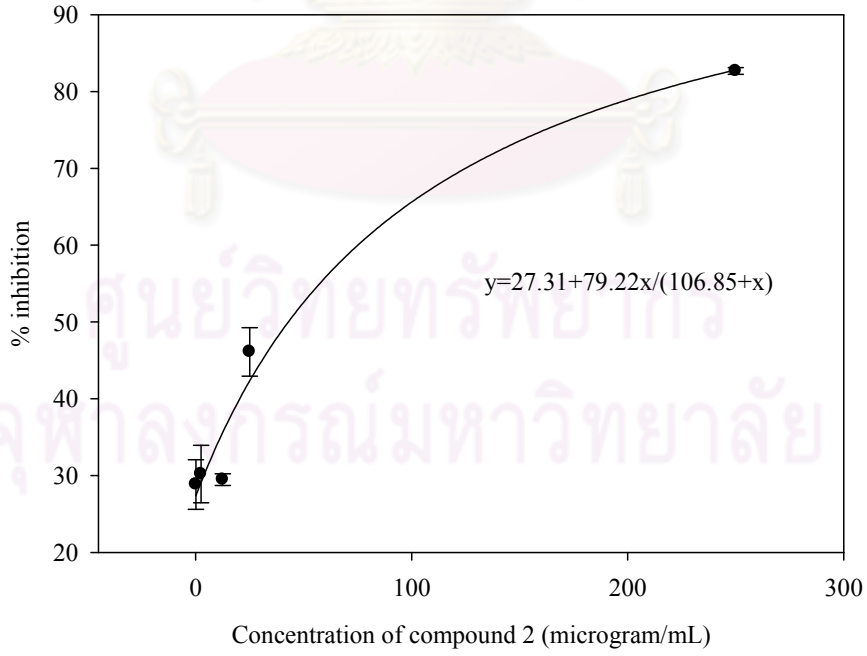


Figure 4.4 Effect of Compound 2 on pancreatic lipase

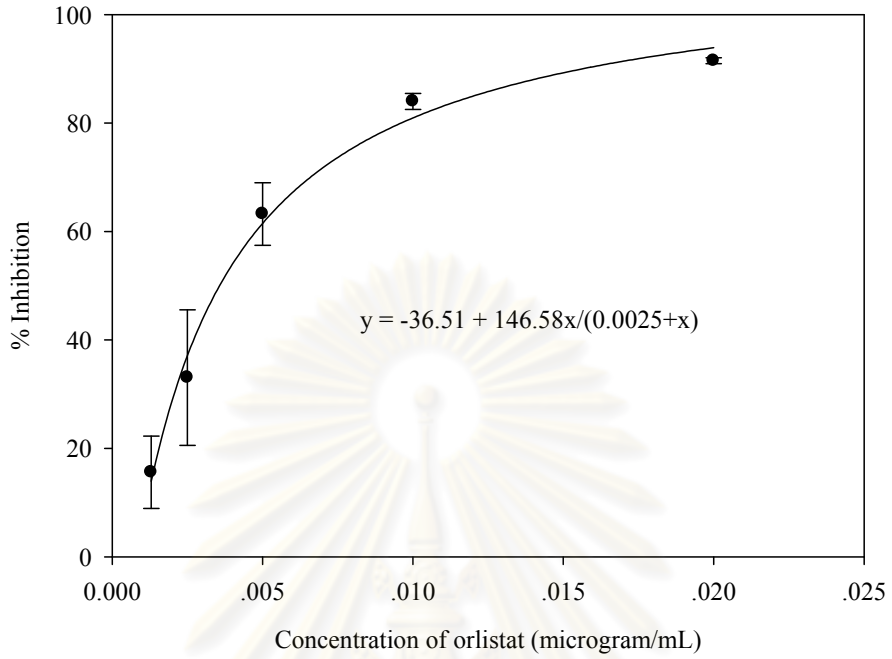


Figure 4.5 Effect of orlistat on pancreatic lipase

Table 4.5 IC_{50} value of orlistat and each isolated compound

Samples	IC_{50}*	
	(µg/ml)	µM
Compound 1	56.0	99.6
Compound 2	43.6	156
Orlistat	0.0035	0.0071

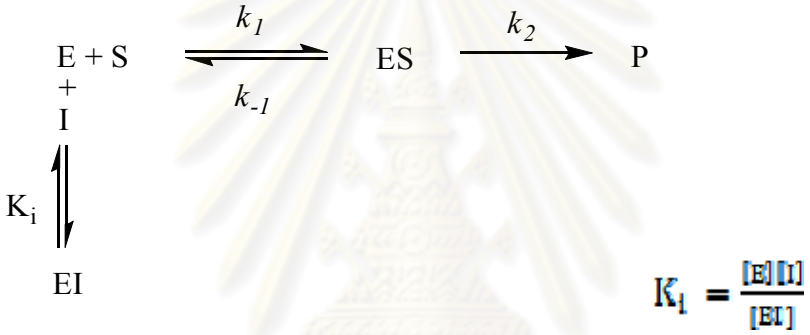
*IC_{50} is the concentration of compound required to give 50% inhibition of lipase activity

Table 4.5 indicated the IC_{50} value of Compound 1, Compound 2 and orlistat as 56.0, 43.6 and 0.0035 µg/ml, respectively. Compound 1 and 2 might be low inhibitory activity when compared with anti-obesity drug (orlistat) which is a derivertive of lipstatin from microbial sources (*S. toxytricini*). From chapter II (theoretical), it shows that lipase inhibitor from microbial sources exhibit inhibitory activity more than that extracted [12, 40-77] from plants. When comparing lipase inhibitory activity of isolated compounds, Compound 1 and 2, with other lipase inhibitor that extracted from other plants [12, 40-66]. They exhibit high inhibitory

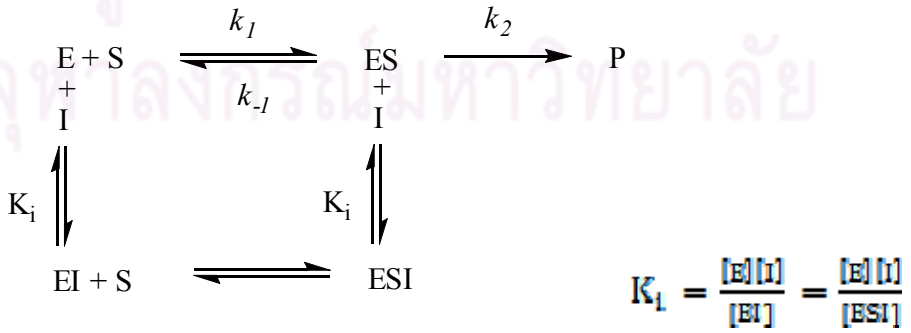
activity. For example, sessiloside, lipase inhibitor that extracted from the leaves of *Accanthopanax sessiliflorus*, showed strong inhibition of pancreatic lipase in vitro, its IC_{50} values was 360 µg/ml.

4.4 Investigation of inhibitory properties of the isolated compounds

The Lineweaver-Burk plot of Compound 1 indicated that mechanism of inhibition of Compound 1 was a competitive inhibition because the kinetic parameters, it was found that K_m increased while V_{max} was constant. , as shown in Figure 4.6-4.7. The reaction scheme of competitive inhibition has shown below.



The Lineweaver-Burk plot of Compound 2 indicated that mechanism of inhibition of Compound 2 was a noncompetitive inhibition because every concentration of Compound 2 had the same X-intercepts in Lineweaver-Burk plot and had potent inhibitory activity, as shown in Figure 4.8-4.9. The reaction scheme of noncompetitive inhibition has shown below.



The K_i (dissociation constant for the EI or ESI complex values of Compound 1 and 2 were determined from the slope of each graph in Lineweaver-Burk plot. The equation for calculation K_i value of competitive and noncompetitive inhibitor are

$$\text{Slope} = K_m/V_{max}[1+([I_0]/K_i)]$$

The K_i values of isolated compounds are shown in Table 4.6.

Table 4.6 The K_i values of isolated compound

Inhibitor	Inhibitor concentration (µg/mL)	V_{max} (U/mg protein)	K_m (mM^{-1})	K_i (µg/mL)
No inhibitor	0	0.083	0.095	-
Compound 1	2.5	0.096	0.435	0.855
	12.5	0.060	0.466	2.175
	25	0.061	0.784	2.470
	125	0.064	1.346	7.281
Compound 2	2.5	0.023	0.099	0.921
	12.5	0.017	0.101	2.981
	25	0.011	0.098	3.817
	125	0.007	0.099	11.356

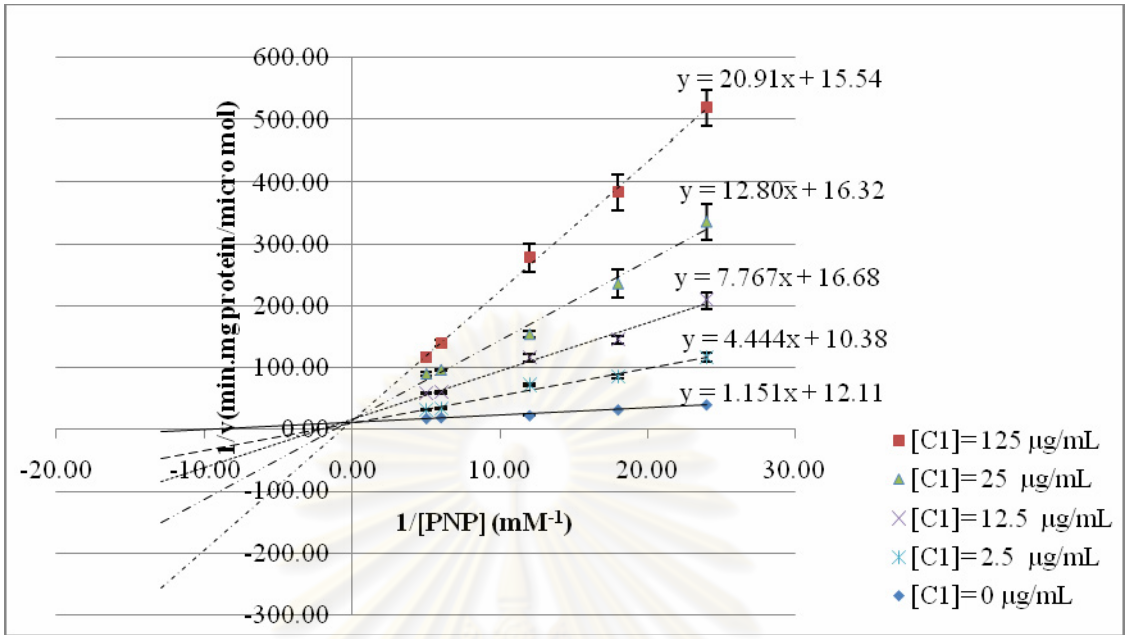


Figure 4.6 Linewaveaver-Burk plot analysis of the inhibition kinetics of lipase by Compound 1 ([C1] = concentration of compound 1)

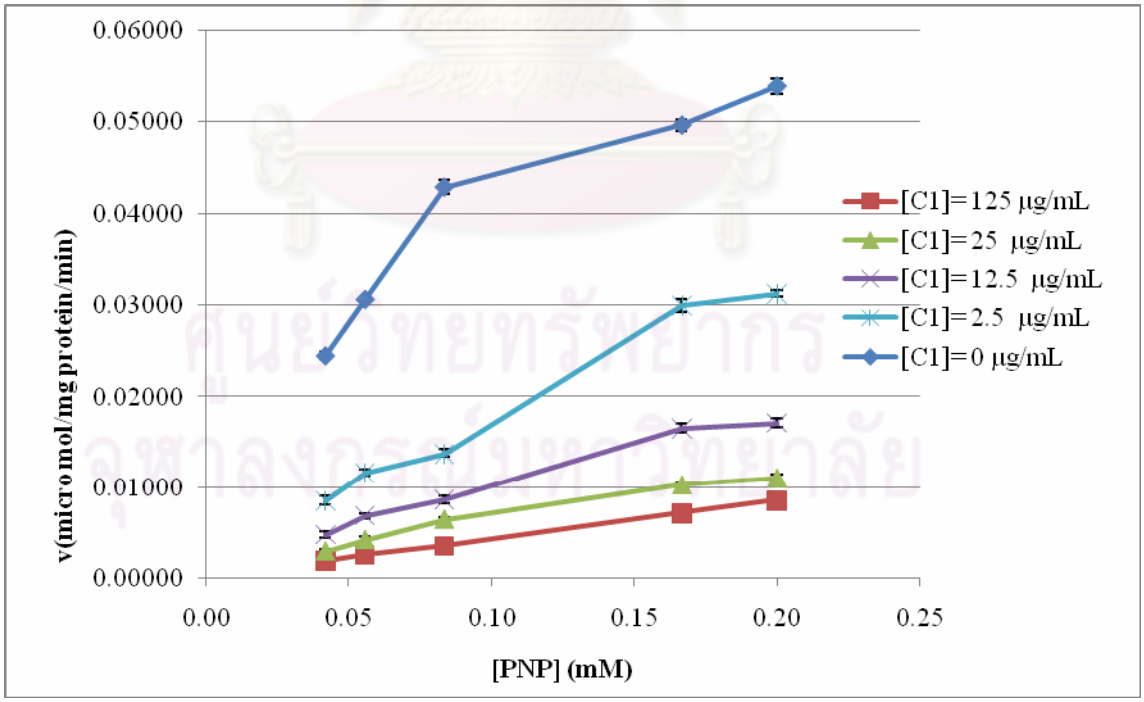


Figure 4.7 Michaelis-Menten plot analysis of the inhibition kinetics of lipase by Compound 1 ([C1] = concentration of compound 1)

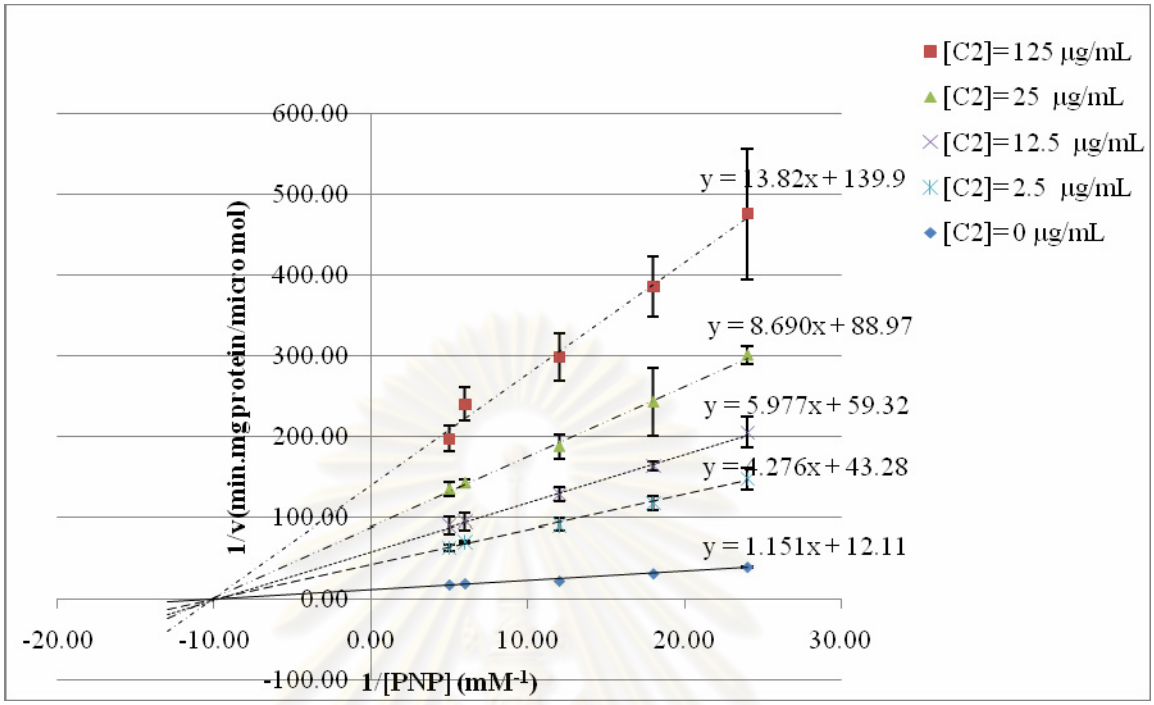


Figure 4.8 Linewaveaver-Burk plot analysis of the inhibition kinetics of lipase by Compound 2 ([C2] = concentration of compound 2)

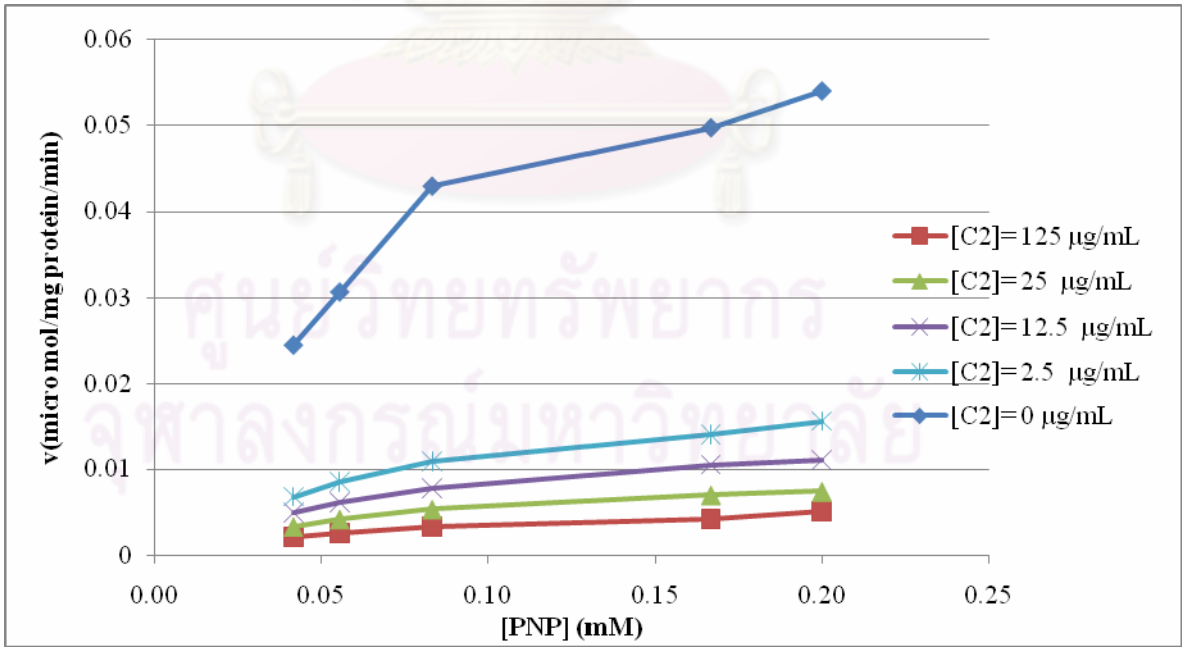


Figure 4.9 Michaelis-Menten plot analysis of the inhibition kinetics of lipase by Compound 2 ([C2] = concentration of compound 2)

4.5 Determination of efficacy of combination of orlistat with isolated compounds

In terms of efficacy, there can be three types of interactions between medications: additive, synergistic, and antagonistic. Figure 4.8 and 4.9 indicated that types of interactions between orlistat with Compound 1 and 2 were antagonistic interaction. Antagonistic interaction means that the effect of two chemicals is actually less than the sum of the effect of the two drugs taken independently of each other.

Table 4.7 Percentage inhibition of orlistat and the combination of orlistat with isolated Compound 1

Orlistat concentration (µg/L)	% Enzyme inhibition		
	Orlistat	Orlistat + Compound 1 (6.25 µg/ml)	Compound 1 (6.25 µg/ml)
0.05	90.5 ± 6.3	79.9 ± 1.8	13.2 ± 10.5
0.025	69.2 ± 9.9	60.0 ± 14.0	
0.01	43.1 ± 5.2	32.6 ± 8.5	
0.0025	33.4 ± 6.9	19.7 ± 6.3	
0.00125	11.4 ± 7.9	5.6 ± 2.9	

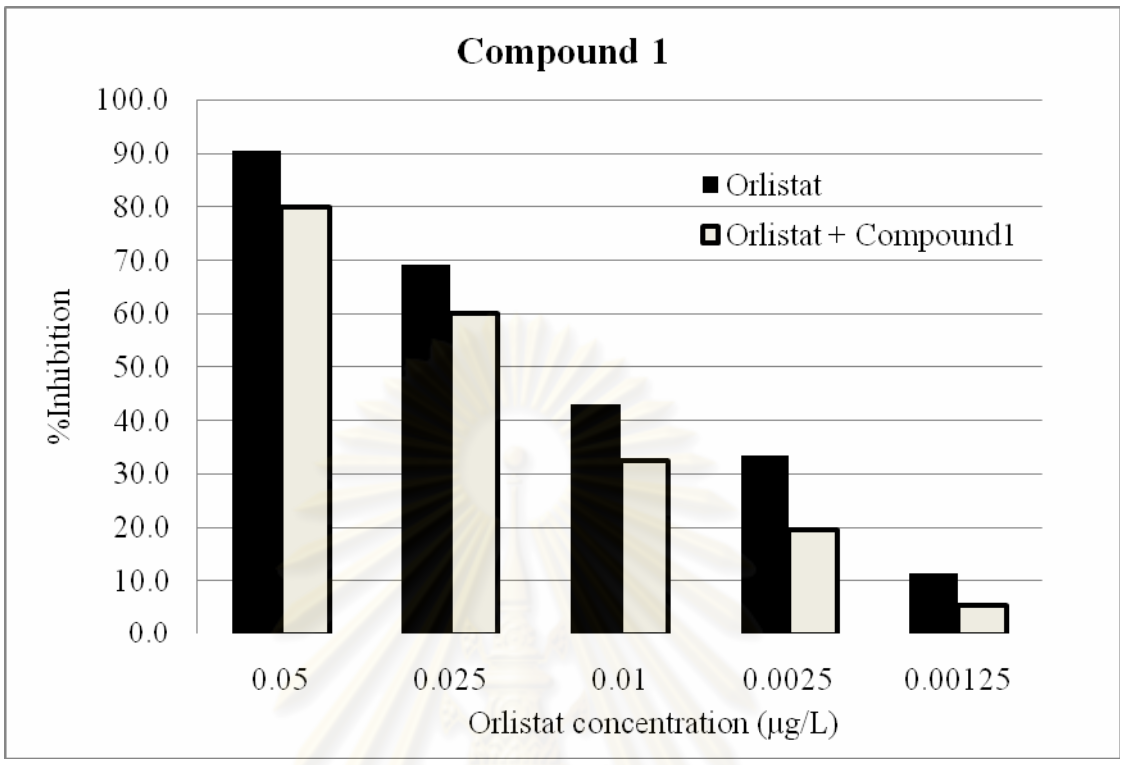


Figure 4.10 The efficacy of combination of orlistat with isolated Compound 1

Table 4.8 Percentage inhibition of orlistat and the combination of orlistat with isolated Compound 2

Orlistat concentration (µg/L)	% enzyme inhibition		
	Orlistat	Orlistat + Compound 2 (6.25 µg/ml)	Compound 2 (6.25 µg/ml)
0.05	90.5 ± 6.3	58.9 ± 2.4	23.9 ± 0.9
0.025	69.2 ± 9.9	53.8 ± 8.7	
0.01	43.1 ± 5.2	34.1 ± 2.8	
0.0025	33.4 ± 6.9	17.9 ± 2.8	
0.00125	11.4 ± 7.9	1.9 ± 9.3	

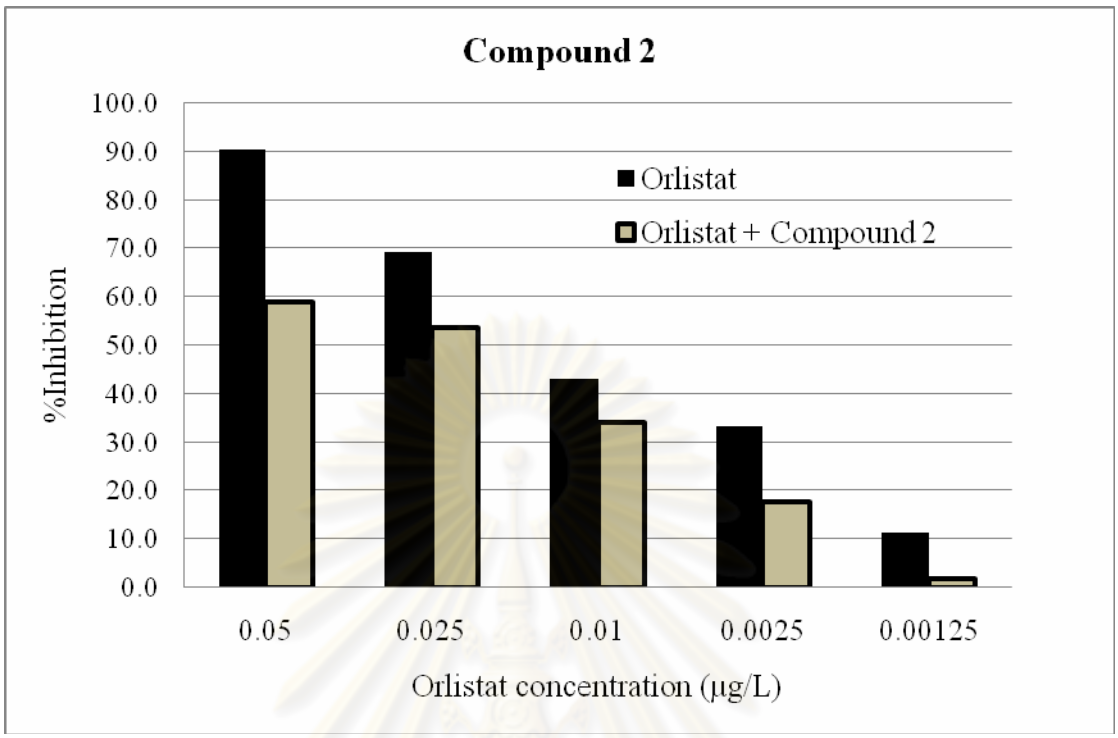


Figure 4.11 The efficacy of combination of orlistat with isolated Compound 2

4.6. Structure elucidation of isolated compounds

4.6.1 Structure elucidation of Compound 1

Properties of compound 1

Compound 1 is white pellet crystal (1.1516 g, 0.09% w/w of the dried fruits) Rf = 0.41(Ethyl acetate : hexane (4:7 v/v)) mp225 °c $[\alpha]_D^{25}$ +41.6(CH_2Cl_2; c 0.2)

Structure elucidation of compound 1

The IR spectrum of Compound 1 (Figure 5.1) displayed broad band at 3453 cm^{-1} belonging to O-H stretching and the absorption peak of C=O stretching vibration at1705 cm^{-1}, C-O stretching vibration at1273 $cm^{-1}$and C=C stretching vibration of aromatic at1450-1600 cm^{-1}. The IR spectrum of Compound 1 was summarized in Table 4.9

Table 4.9 The IR absorption bands assignment of Compound 1

Wave number (cm^{-1})	Peak intensity	Tentative assignment
3453	Broad	O-H stretching vibration of alcohol
3035-3091	Medium, sharp	C-H stretching vibration of alkenyl
2960,2871	Strong	C-H stretching vibration of alkenyl
1705	Strong	C=O stretching vibration
1450-1600	Weak to strong	C=C stretching vibration of aromatic
1273	Strong	C-O stretching vibration

The ^{1}H-NMR spectrum (Figure 5.2) exhibitd the important signal at δ 5.7 ppm which compatible with alkylene proton (CR_2=CRH) . A proton of aromatic show the signal at δ 8.05 , 7.56 and 7.45 ppm. A proton of H-C-O show the signal δ 4.7 ppm. Other in the region of δ 3.73 ppm were attributed to proton of O=C-H.

The ^{13}C-NMR spectrum (Figure 5.3) displayed a total 35 signals of carbon signals of carbon. It showed aromatic carbon signals at δ 130.6, 129.6, 128.4 and 133 ppm. The carboxylic carbon showed the signals at δ 166.5 ppm. The signal of carbonyl carbon were also evidenced at δ 200 ppm

The EI mass spectrum (Figure 5.4) of this compound gave a molecular ion at m/z 562 which was corresponding to the molecular formula $C_{37}H_{54}O_4$. These NMR data precluded the possibility of steroid containing benzoyl group, one hydroxylgroup, one carbonyl group and one double bond. Its structure could be deduced as carpesterol. This deduction was established by comparing the ^{13}C-NMR spectrum of this compound with those of the known carpesterol [80]. The result was displayed in Table 4.10

Table 4.10 The ^{13}C-NMR chemical assignment of Compound 1[80]

Carbon position	Chemical shift (δ ppm)	
	Compound 1	Capesterol
C-1	36.3	36.3
C-2	26.3	26.2
C-3	79.1	79.0
C-4	31.9	31.8
C-5	60.1	60.0
C-6	200.3	200.2
C-7	123.7	123.7
C-8	161.1	161.0
C-9	51.1	51.1
C-10	39.4	39.3
C-11	21.8	21.7
C-12	38.8	38.8
C-13	45.2	45.1
C-14	55.0	55.0
C-15	22.7	22.6
C-16	27.1	27.0
C-17	53.2	53.1
C-18	12.4	12.4
C-19	14.8	14.7
C-20	42.7	42.6
C-21	12.6	12.5
C-22	71.2	71.0
C-23	30.1	30.0
C-24	41.5	41.4
C-25	28.8	28.8
C-26	17.7	17.6
C-27	20.6	20.5
C-28	23.7	23.6
C-29	11.9	11.8
C-30	17.6	17.5
C-31	166.5	166.5
C-1’	130.6	130.5
C-2’, C-6’	129.7	129.6
C-3’ ,C-5’	128.4	128.4
C-4’	133.0	132.9

It could be concluded that Compound 1 exhibited the ^{13}C-NMR chemical shifts similarly to those of carpesterol. Therefore, Compound 1 was assigned as carpesterol which was previously isoate from *S. sisymbrifolium*[80], *S. xanthocarpum* [81-82] *and S. indicum* [83].

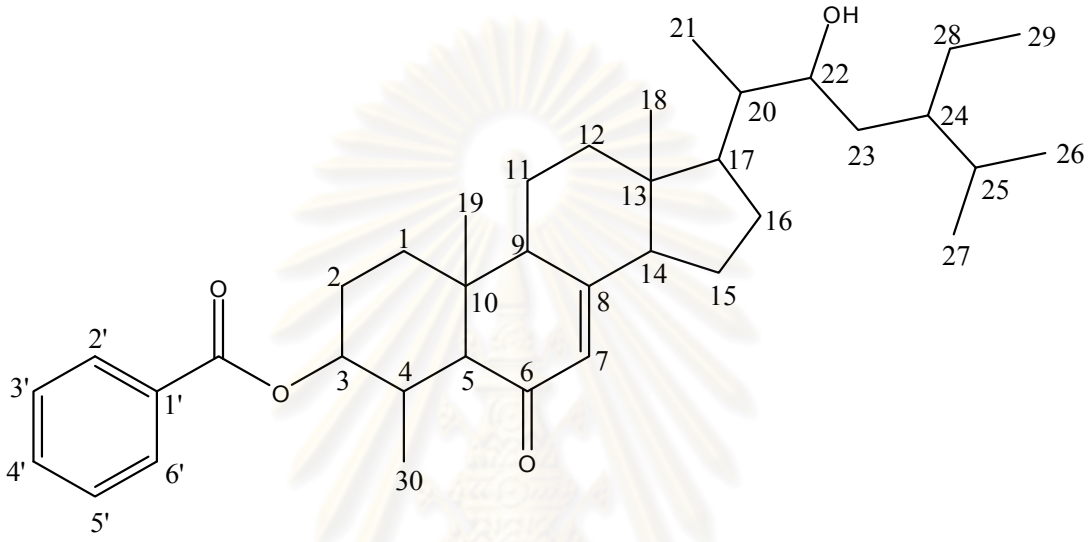


Figure 4.12 The structure of compound 1

4.6.2 Structure elucidation of compound 2

Properties of Compound 2

Compound 2 was slightly yellow wax (1.7250 g, 0.13% w/w of the dried fruits) Rf = 0.55 (Ethyl acetate : hexane (4:7 v/v)) mp 48 °c $[\alpha]_D^{25}$ +1.115 (CH_2Cl_2; c 0.2)

Structure elucidation of Compound 2

The IR spectrum of compound 2 (Figure 5.9) displayed broad band at 3000-3500 cm^{-1} belonging to O-H stretching and the absorption peak of C=O stretching vibration at1700 cm^{-1} and C-O stretching vibration at 1320 cm^{-1}. The IR spectrum of Compound 2 was summarized in Table 4.11.

Table 4.11 The IR absorption bands assignment of Compound 2

Wave number (cm^{-1})	Peak intensity	Tentative assignment
3000-3500	Broad	O-H stretching vibration of alcohol
2900	Strong	C-H stretching vibration
1700	Strong	C=O stretching vibration
1320	strong	C-O stretching vibration
1400,950	Strong	O-H bending vibration

The ^{1}H-NMR spectrum (Figure 5.10) exhibitd the important signal at δ 5. 35 ppm. which compatible with alkylene proton (CRH=CRH) . A proton of terminal methyl proton showed the signal at δ 0.89 ppm.

The ^{13}C-NMR spectrum (Figure 5.11) displayed a total 18 signals of carbon, one carbonyl carbon signal at δ 180, four olefinic methine carbons at δ 127.9, 128.0, 130.0 and 130.2, twelve methylene carbons, one terminal methyl carbon at δ 14.0.

The FAB^{+} mass spectrum (Figure 5.12) of this compound gave a molecular ion at m/z 280 which was corresponding to the molecular formula $C_{18}H_{32}O_2$. These NMR data indicated the possibility of long chain unsaturated fatty acid. Its structure could be deduced as linoleic acid. This deduction was established by comparing the ^{13}C-NMR spectrum of this compound with those of the known linoleic acid [85]. The result was displayed in Table 4.12.

Table 4.12 The ^{13}C-NMR chemical assignment of compound 2 [84]

Carbon position	Chemical shift (ppm)	
	Compound 2	Linoleic acid
C-1	180.0	170.0
C-2	33.9	34.1
C-3	24.6	24.7
C-4	29.0-29.3	29.0–29.1
C-5	29.0-29.3	29.0–29.1
C-6	29.0-29.3	29.0–29.1
C-7	29.6	29.6
C-8	27.2	27.5
C-9	130.0	131.9
C-10	128.0	123.7
C-11	127.8	123.6
C-12	130.2	132.2
C-13	27.2	27.4
C-14	29.7	29.6
C-15	29.0-29.3	29.0–29.1
C-16	31.9	31.8
C-17	22.7	22.6
C-18	14.0	14.1

It could be concluded that Compound 2 exhibited the ^{13}C-NMR chemical shifts similarly to those of linoleic acid. Therefore, compound 2 was assigned as linoleic acid which was previously isolated from sunflower seed [85].

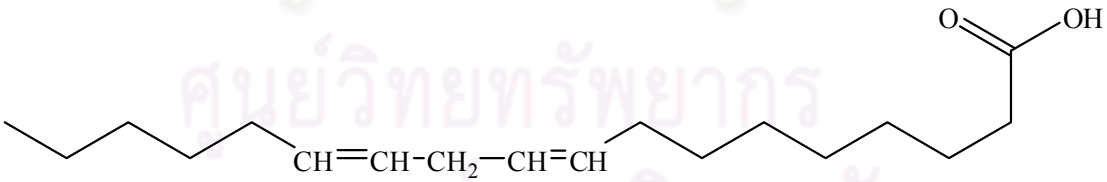


Figure 4.13 The structure of compound 2

CHAPTER V

CONCLUSION

The lipase inhibitor from fruits of *S. stramonifolium* was studied. The fruits of *S. stramonifolium* were extracted by hexane, ethyl acetate and water, respectively to give the crude extracts as green-yellow oil, dark-brown oil and dark-brown gummy, respectively. Then the crude extracts were further assayed for their lipase inhibitory activity at concentration 1.25 mg/mL, to obtain 33.4 ± 2.7, 94.6 ± 8.3 and 1.1 ± 1.5% inhibitions, respectively. Therefore, the hexane and ethyl acetate crude extract were purified by silica-gel column chromatography with hexane-ethyl acetate gradient solvent system to give Compound 1, as white pellet crystal, and Compound 2 as slightly yellow wax. Compound 1 and 2 exhibited high inhibitory activity with IC_{50} 56.00 and 43.60 µg/ml, respectively while orlistat (positive control) showed IC_{50} 0.0035 µg/ml. For investigation of inhibitory properties of the active compounds were found that Compounds 1 was a competitive inhibitor whereas Compound 2 was a noncompetitive inhibitor of porcine pancreatic lipase with the K_i values of 2.17 and 2.98 µg/ml at concentration 12.50 µg/ml, respectively. In terms of efficacy of combination of orlistat with Compound 1 and 2 it was found that they were antagonistic interactions. Compound 1 and 2 were assigned as carpesterol and linoleic acid

Suggestion for future work

Since the lipase in *S. stramonifolium* was carpesterol, therefore quantity of carpesterol in other *Solanum spp.*should be investigated. Moreover, benzoate derivative of other triterpenoids should be studied.

REFERENCES

[1] Cairns, E. 2005. Obesity: the fat lady sings?. Drug Discov. Today. 10 : 305–307.

[2] Foster-Schubert, K. and Cummings, D. 2006. Emerging therapeutic strategies for obesity. Endocr Rev. 27 : 779–793.

[3] Mukherjee, M. 2003. Human digestive and metabolic lipases—a brief review. J. Mol. Catal., B Enzym, 22 : 369–376.

[4] Yamamoto, M., Shimura, S., Itoh, Y., Ohsaka, T., Egawa, M. and Inoue, S. 2000. Anti-obesity effects of lipase inhibitor CT-II, an extract from edible herbs, *Nomame herba*, on rats fed a high-fat diet. Int J Obes. 24 : 758-766.

[5] Han, L., Kimura, Y. and Kawashima, M. 2001. Anti-obesity effects in rodents of dietary tea saponin, a lipase inhibitor. Int J Obes Rel Metab Dis. 25 : 1459-1465.

[6] Yoshikawa, M., Shimoda, H., Nishida, N., Takada, M. and Matsuda, H. 2002. *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. J Nutr. 132 : 1819-1829.

[7] วาทินี จันมี และ ศราวลี ธนศิลป์. 2549. การคัดกรองสารที่มีฤทธิ์ยับยั้งเอนไซม์ไลเพสจากพืชสมุนไพร. โครงการการเรียนการสอนเพื่อเสริมประสบการณ์ ระดับปริญญาวิทยาศาสตรบัณฑิต ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย.

[8] Mayes, P.A. 1983. Metabolism of lipids. I. Fatty acids, In Harper's Review of Biochemistry. 19th ed. pp. 201–223.Singapore : Lange Medical Publications.

[9] Mukherjee, M. 1993. Lipoproteins and apolipoproteins—function, metabolism and disorder. Bombay Hosp. J. 35 : 75–82.

[10] Phan, C.T. and Tso, P. 2001. Intestinal lipid absorption and transport. Front. Biosci. 6 : D299–D319.

[11] Choi, S.Y., Hirata, K., Ishida, T., Quertermous, T. and Cooper, A.D. 2002. Endothelial lipase : a new lipase on the block. J. Lipid Res. 43 : 1763–1769.

[12] Birari, R. B. and Bhutani, K. K., 2007. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discovery Today. 12 : 879-889.

[13] Hamosh, M. 1990. Lingual and gastric lipase. Nutrition**.** 6 : 421–428.

[14] Moreau, H., Laugier, R., Gargouri, Y., Ferrato, F. and Verger, R. 1988. Human pre-duodenal lipase is entirely of gastric fundic origin. Gastroenterology. 95 :1221–1226.

[15] Lohse, P., Lohse, P., Chahrokh-Zadeh, S. and Seidel, D. 1997. The acid lipase gene family : three enzymes, one highly conserved gene structure. J. Lipid Res. 38 : 880–891.

[16] Bodmer, M.W. *et al.* 1987. Molecular cloning of human gastric lipase and expression of the enzyme in yeast. Biochim. Biophys. Acta. 909 : 237–244.

[17] Bernbaeck, S. and Blaeckberg, L. 1989. Human gastric lipase. The N-terminal tetrapeptide is essential for lipid binding and lipase activity. Eur. J. Biohem. 182 : 495–499.

[18] Hide, W.A., Chan, L. and Li, W.H. 1992. Structure and evolution of the lipase superfamily. J. Lipid Res. 33 : 167–178.

[19] Kirchgessner, T.G., Svenson, K.L., Lusis, A.J. and Schotz, M.C. 1987. The sequence of cDNA encoding lipoprotein lipase. A member of a lipase gene family. J. Biol. Chem. 262 : 8463–8466.

[20] Mukherjee, M. 2003. Human digestive and metabolic lipases—a brief review. J. Mol. Catal., B Enzym. 22 : 369–376.

[21] Shi, Y. and Burn, P. 2004. Lipid metabolic enzymes: Emerging drug targets for the treatment of obesity. Nat. Rev. Drug Discov. 3 : 695–710.

[22] Thomson, A.B., De Pover, A., Keelan, M., Jarocka-Cyrta, E., Clandinin, M.T. 1997. Inhibition of lipid absorption as an approach to the treatment of obesity. In: B. Rubin and E.A. Dennis, Editors, Methods in Enzymology. Academic Press : 3–41.

[23] Winkler, F.K., D'Arcy, A., and Hunziker, W. 1990. Structure of human pancreatic lipase. Nature. 343 : 771–774.

[24] Tilbeurgh, H. V., Sarda, L., Verger, R. and Cambillau, C. 1992. Structure of the pancreatic lipase-procolipase complex. Nature. 359 : 159–162.

[25] Kazlauskas, R.J. 1994. Elucidating structure-mechanism relationships in lipases: Prospects for predicting and engineering catalytic properties. TIBTECH. 12 : 464–472

[26] Gargouri, Y., Ransac, S., and Verger R., 1997. Covalent inhibition of digestive lipases: an in vitro study. Biochimica et Biophysica Acta. 1344 : 6-37

[27] RCP. 2005. Anti-obesity drugs. Guidance on appropriate prescribing and management. London: Royal College of Physicians of London.

[28] Hofbauer, K.G. 2002. Molecular pathways to obesity. Int. J. Obes. 26 : S18–S27.

[29] Srivastava, R.K. and Srivastava, N. 2004. Search for obesity drugs: Targeting central and peripheral pathways. Curr. Med. Chem. – Immun., Endoc. & Metab. Agents. 4 : 75–90.

[30] Weigle, D.S. 2003. Pharmacological therapy of obesity: Past, present, and future. J. Clin. Endocrinol. Metab. 88 : 2462–2469.

[31] Foster-Schubert, K.E. and Cummings, D.E. 2006. Emerging therapeutic strategies for obesity. Endocr. Rev. 27 : 779–793.

[32] Strader, C.D., Hwa, J.J., Van Heek, M. and Parker E.M. 1998. Novel molecular targets for the treatment of obesity. Drug Discov. Today. 3 : 250–256.

[33] Bitou, N., Ninomiya, M., Tsujita, T. and Okuda, H. 1999. Screening of lipase inhibitors from marine algae. Lipids. 34 : 441–445.

[34] Weibel, E.K., Hadvary, P., Hochuli, E., Kupfer, E. and Lengsfeld, H. 1987. Lipstatin, an inhibitor of pancreatic lipase, produced by *Streptomyces toxytricini*. I. Producing organism, fermentation, isolation and biological activity. J. Antibiot. 40 : 1081–1085.

[35] Zhi, J., Mulligan, T. E. and Hauptman, J. B. 1999. Long-term systemic exposure of orlistat, a lipase inhibitor, and its metabolites in obese patients. J. Clin. Pharmacol. 39, 41–46.

[36] Hadvary, P., Sidler, W., Meister, W., Vetter, W. and Wolfer, H. 1991. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. J. Biol. Chem. 266 : 2021–2027.

[37] Lucas, C.P., Boldrin, M.N. and Reaven, G. M. 2003. Effect of orlistat added to diet (30% of calories from fat) on plasma lipids, glucose, and insulin in obese patients with hypercholesterolemia. Am. J. Cardiol. 91: 961–964.

[38] Agra, M.F., Freitas, P.F. and Barbosa, F. JM. 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Rev Bras Farmacog. 17 : 116–155.

[39] Hayati, N.E. Sukprakarn, S. and Juntakool, S. 2005. Seed Germination Enhancement in Solanum stramonifolium and Solanum torvum. Kasetsart J. (Nat. Sci.). 39 : 368 – 376

[40] Han, L.K., Xu, B.J., Kimura, Y., Zheng, Y. and Okuda, H. 2000. *Platycodi* Radix affects lipid metabolism in mice with high fat diet-induced obesity. J. Nutr. 130 : 2760–2764

[41] Han, L.K., Zheng, Y.N., Xu, B.J., Okuda, H. and Kimura, Y. 2002. Saponins from *Platycodi* Radix ameliorate high fat diet-induced obesity in mice. J. Nutr. 132 : 2241–2245.

[42] Zao, H.L. and Kim, Y.S. 2004. Determination of the kinetic properties of platycodin D for the inhibition of pancreatic lipase using a 1,2-diglyceride-based colorimetric assay. Arch. Pharm. Res. 27 : 968–972.

[43] Xu, B.J., Han, L.K., Zheng, Y.N., Lee, J.H. and Sung C.K. 2005. *In vitro* inhibitory effect of triterpenoidal saponins from *Platycodi* Radix on pancreatic lipase. Arch. Pharm. Res. 28 : 180–185.

[44] Zhao, H.L., Sim, J-S. and Shim, S.H., 2005. Antiobese and hypolipidemic effects of platycodin saponins in diet-induced obese rats: evidences for lipase inhibition and calorie intake restriction. Int. J. Obes. 29 : 983–990.

[45] Zheng, Q. and Koike, K. 2004. New biologically active triterpenoid saponins from *Scabiosa tschiliensis*. J. Nat. Prod. 67 : 604–613.

[46] Yoshizumi, K. *et al.* 2006. Lupane type saponins from leaves of *Acanthopanax sessiliflorus* and their inhibitory activity on pancreatic lipase. J. Agric. Food Chem. 54 : 335–341.

[47] Han, L.K., Zheng, Y.N., Yoshikawa, M., Okuda, H. and Kimura, Y. 2005. Anti-obesity effects of chikusetsusaponins isolated from *Panax japonicus* rhizomes. BMC Complement. Altern. Med. 5 : 9–18.

[48] Kwon, C.S. *et al.* 2003. Anti-obesity effects of *Dioscorea nipponica* Makino with lipase inhibitory activity in rodents. Biosci. Biotechnol. Biochem. 67 : 1451–1456.

[49] Kimura, H., Ogawa, S., Jisaka, M., Kimura, Y., Katsube, T. and Yokota, K. 2006. Identification of novel saponins from edible seeds of Japanese horse chestnut (*Aesculus turbinata* Blume) after treatment with wooden

ashes and their nutraceutical activity. J. Pharm. Biomed. Anal. 41 : 1657–1665.

[50] Han, L.K., Takaku, T., Li, J., Kimura, Y. and Okuda, H. 1999. Anti-obesity action of oolong tea. Int. J. Obes. 23 : 98–105.

[51] Han, L.K., Kimura, Y. and Kawashima, M. 2001. Anti-obesity effects in rodents of dietary teasaponin, a lipase inhibitor. Int. J. Obes. 25 : 1459–1464.

[52] Kurihara, H. 2003. Hypolipemic effect of *Cyclocarya paliurus* (Batal) Iljinskaja in lipid-loaded mice. Biol. Pharm. Bull. 26 : 383–385.

[53] Masayuki, Y. *et al.* 2002. *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lypophilic activities have mild antiobesity effects in rats. J. Nutr. 132 : 1819–1834.

[54] Yamamoto, M., Shimura, S., Itoh, Y., Ohsaka, T., Egawa, M. and Inoue, S. 2000. Anti-obesity effects of lipase inhibitor CT-II, an extract from edible herbs, Nomame Herba, on rats fed a high-fat diet. Int. J. Obes. 24 : 758–764.

[55] Shin, J.E., Joo, H. M. and Kim DH. 2002. 3-Methylethergalangin isolated from *Alpinia officinarum* inhibits pancreatic lipase. Biol. Pharm. Bull. 25 : 1442–1445.

[56] Kawaguchi, K., Mizuno, T., Aida, K. and Uchino, K. 1997. Hesperidin as an inhibitor of lipases from porcine pancreas and Pseudomonas. Biosci. Biotechnol. Biochem. 61 : 102–104.

[57] Nakai, M. *et al.* 2005. Inhibitory effects of oolong tea polyphenols on pancreatic lipase *in vitro*. J. Agric. Food Chem. 53 : 4593–4598.

[58] Moreno, D.A., Ilic, N., Poulev, A., Brasaemle, D.L., Fried, S.K. and Raskin, I. 2003. Inhibitory effects of grape seed extract on lipases. Nutrition 19 : 876–879.

[59] Ono, Y., Hattori, E., Fukaya, Y., Imai, S., and Ohizumi, Y. 2006. Anti-obesity effect of *Nelumbo nucifera* leaves extract in mice and rats. J. Ethnopharmacol. 106 : 238–244.

[60] Yoshikawa, M., Shimoda, H., Nishida, N. and Takeda, M.2002. *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lypophilic activities have mild antiobesity effects in rats. J. Nutr. 132 : 1819–1834

[61] Moreno, D.A., Ilic, N., Poulev, A. and Raskin, I. 2006. Effects of *Arachis hypogaea* nutshell extract on lipid metabolic enzymes and obesity parameters. Life Sci. 78 : 2797–2803.

[62] Moreno, D.A., Ripoll, C., Ilic, N., Poulev, A., Aubin, C. and Raskin, I. 2006. Inhibition of lipid metabolic enzymes using *Mangifera indica* extracts. J. Food Agric. Environ. 4 : 21–26

[63] Hatano, T. *et al.* 1997. Flavan dimers with lipase inhibitory activity from *Cassia nomame*, Phytochemistry. 46 : 893–900.

[64] K. Ninomiya *et al.*, 2004. Carnosic acid, a new class of lipid absorption inhibitor from sage. Bioorg. Med. Chem. Lett. 14 : 1943–1946.

[65] Lee, I.A., Lee, J.H., Baek, N.I. and Kim, D.H.2005. Antihyperlipidemic effect of crocin isolated from the fructus of *Gardenia jasminoides* and its metabolite crocetin. Biol. Pharm. Bull. 28 : 2106–2110.

[66] Sheng, L., Qian, Z., Zheng, S. and Xi, L.2006. Mechanism of hypolipidemic effect of crocin in rats: Crocin inhibits pancreatic lipase. Eur. J. Pharmacol. 543 : 116–122.

[67] Weibel, E.K., Hadvary, P., Hochuli, E., Kupfer, E. and Lengsfeld, H.1987. Lipstatin, an inhibitor of pancreatic lipase, produced by S*treptomyces toxytricini* I. Producing organism, fermentation, isolation and biological activity. J. Antibiot. XL : 1081–1085.

[68] Hochuli, E., Kupfer, E., Maurer, R., Meister, W., Mercadal, Y. and Schmidt, K.1987. Lipstatin, an inhibitor of pancreatic lipase, produced by S*treptomyces toxytricini* II. Chemistry and structure elucidation. J. Antibiot. XL : 1086–1091.

[69] Mutoh, M. *et al.* 1994. Panclicins, novel pancreatic lipase inhibitors I. Taxonomy, fermentation, isolation and biological activity. J. Antibiot. 47 : 1369–1375.

[70] Yoshinari, K. *et al.* 1994. Panclicins, novel pancreatic lipase inhibitors II. Structural elucidation. J. Antibiot. 47 : 1376–1384.

[71] Kltahara, M. *et al.* 1987. Valilactone, an inhibitor of esterase, produced by actinomycetes. J. Antibiot. XL : 1647–1650.

[72] Umezawa, H., Aoyagi, T., Uotani, K., Hamada, M., Takeuchi, T. and Takahashi S. 1980. Ebelactone, an inhibitor of esterase, produced by actinomycetes. J. Antibiot. XXXIII : 1594–1596.

[73] Umezawa, H. *et al.* 1978. Esterastin, an inhibitors of esterase produced by actinomycetes. *J. Antibiot.* XXXI : 639–641.

[74] Tomoda, H., Namatame, I., and Omura S. 2002. Microbial metabolites with inhibitory activity against lipid metabolism. Proc. Japan Acad. 78 : 217–240.

[75] Dong, L. *et al.* 2006. Vibralactone: a lipase inhibitor with an unusual fused-lactone produced by cultures of the *Basidiomycete Boreostereum vibrans*. Org. Lett. 8 : 5749–5752.

[76] Cordula, H., Michael, K., Guenter, M. and Luigi, T. 2001. Aventis Pharma. Percyquinnin, a process for its production and its use as a pharmaceutical. 6596518.

[77] Bitoua, N. *et al.* 1999. Screening of lipase inhibitors from marine algae. Lipids : 441–445.

[78] Slanc, P., Doljak, B., Mlinaric, A., Strukelj, B. 2004. Screening of wood damaging fungi and macrofungi for inhibitors of pancreatic lipase. Phytotherapy. 15 : 758-762.

[79] Lowry, O. H., Rosebrough, N. J., Farr, A.L. and Randall, R. J., 1951. Protein measurement with the Folin-Phenol reagents. J. Biol. Chem. 193: 265-275.

[80] Chakravarty, A.K., Mukhopadhyay, S., Saha, S. and Pakrashi, S.C. 1996. A neolignan and sterols in fruits of *Solanum sisymbrifolium.* Phytochemistry. 41 : 935-939.

[81] Singh, O.M., Subharani, K. Singh, N. I., Devi, N. B. and Nevidita L. 2007. Isolation of steroidal glycosides from *Solanum xanthocarpum* and studies on their antifungal activities . Nat Prod Res. 21 : 585-590

[82] Kusano, G., Kusano, G., Takemoto, T., Beisler, J.A., and Sato, Y. 1975. Steroidal constituents of Solanum xanthocarpum. Phytochemistry 14 : 529-532.

[83] Beisler, J. A. and Sato, Y. 1971. The Chemistry of Carpesterol, a Novel Sterol from *Solanum xanthocarpum.* J . Org. Chem. 36 : 3946 – 3950.

[84] Davis, A.L., McNeill, G.P. and Caswell, D.C. 1999. Analysis of conjugated linoleic acid isomers by ^{13}C NMR spectroscopy. Chem. Phys. Lipids 97 : 155-165.

[85] Jasso, R.D., Phillips, B.S., Rodriguez, G.R. and Angulo, S.J.L. 2002. Trends in New Crops and New Uses. In J. Janick and A. Whipkey (eds.) Grain yield and fatty acid composition of sunflower seed for cultivars developed under dry land conditions. pp. 139-142. USA : ASHS Press.

APPENDIX

Figure 5.1 IR spectrum of Compound 1

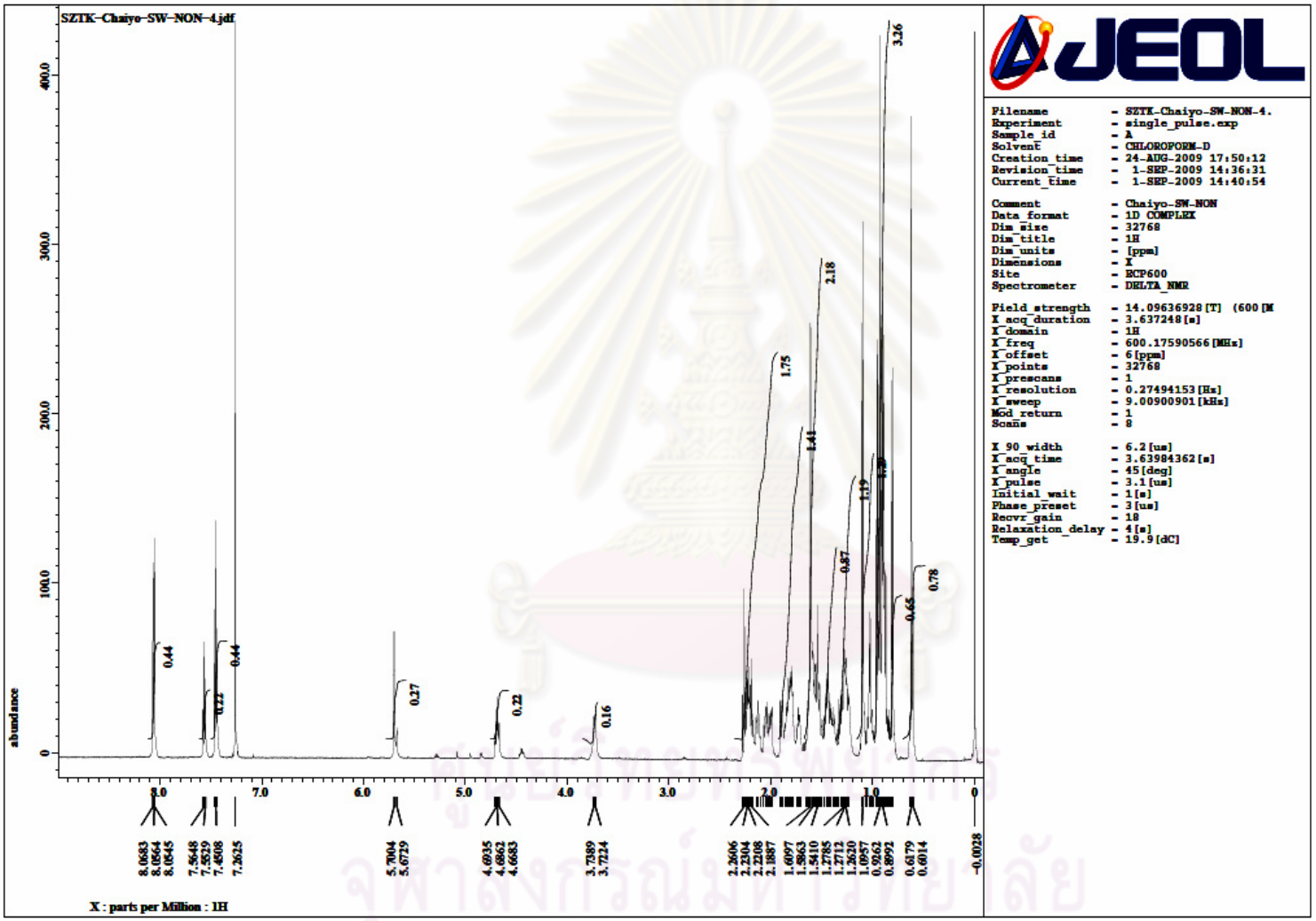


Figure 5.2 ^{1}H-NMR of Compound 1

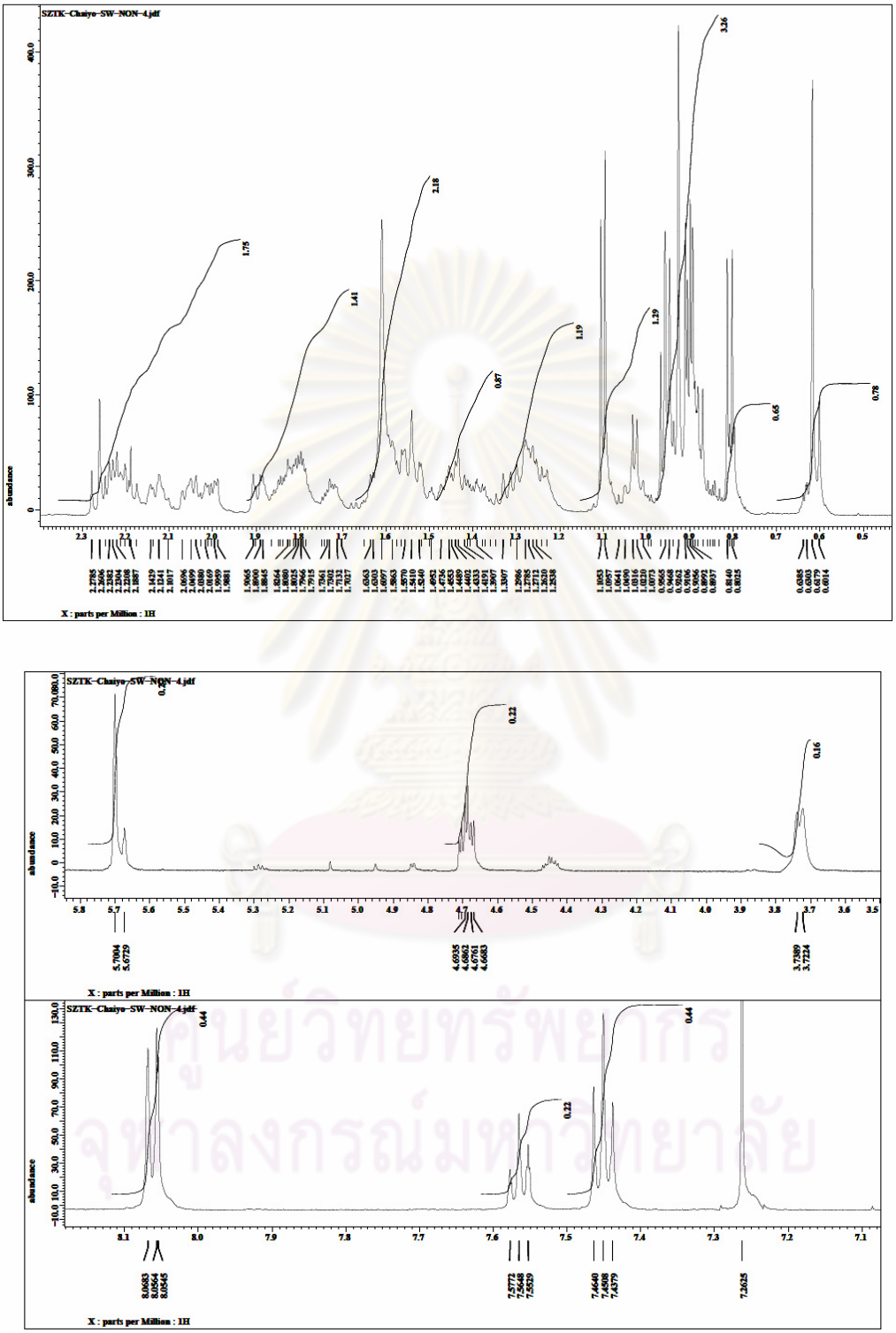


Figure 5.2 ^{1}H-NMR of Compound 1 (expand)

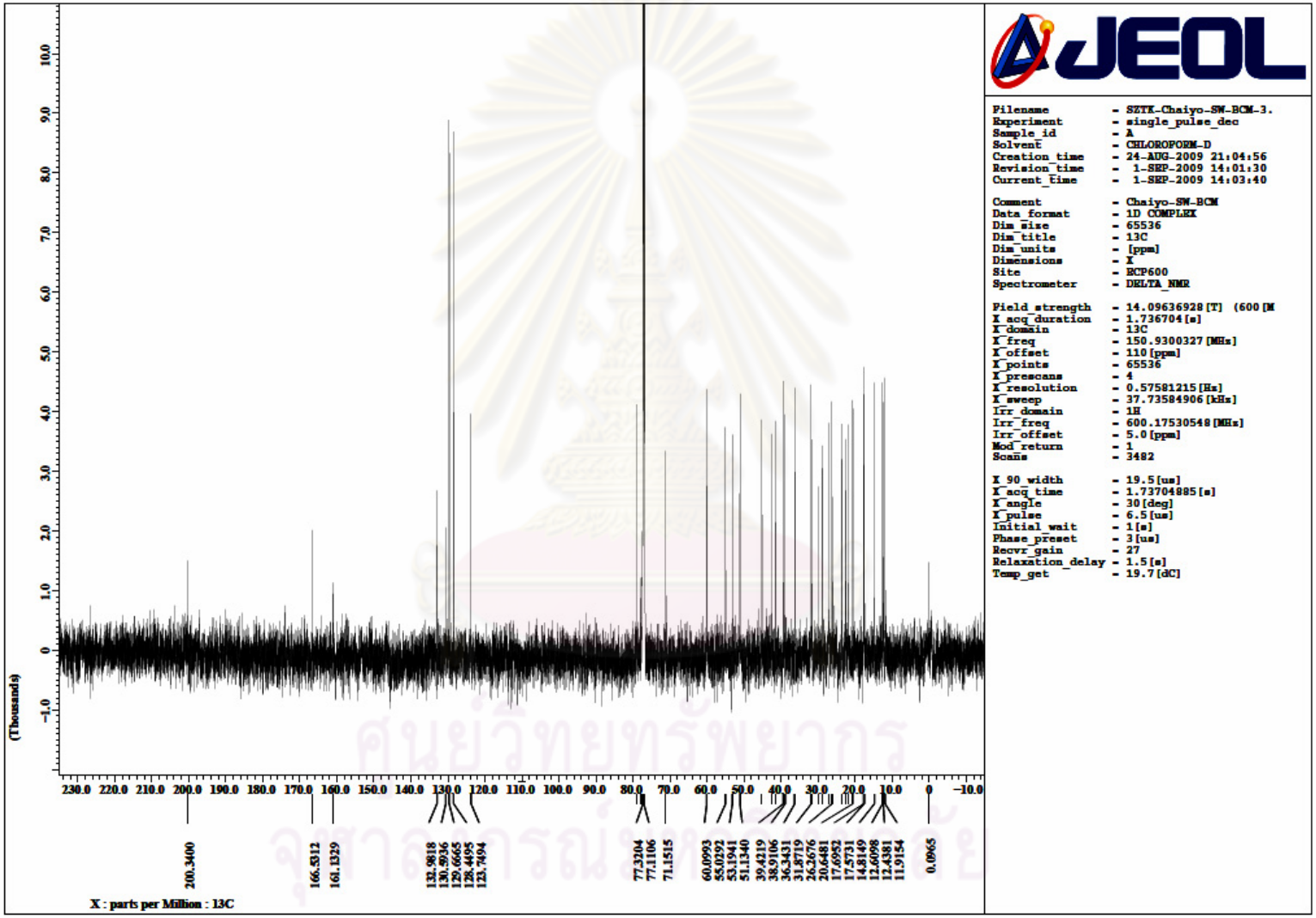


Figure 5.3 **^{13}C-NMR of Compound 1**

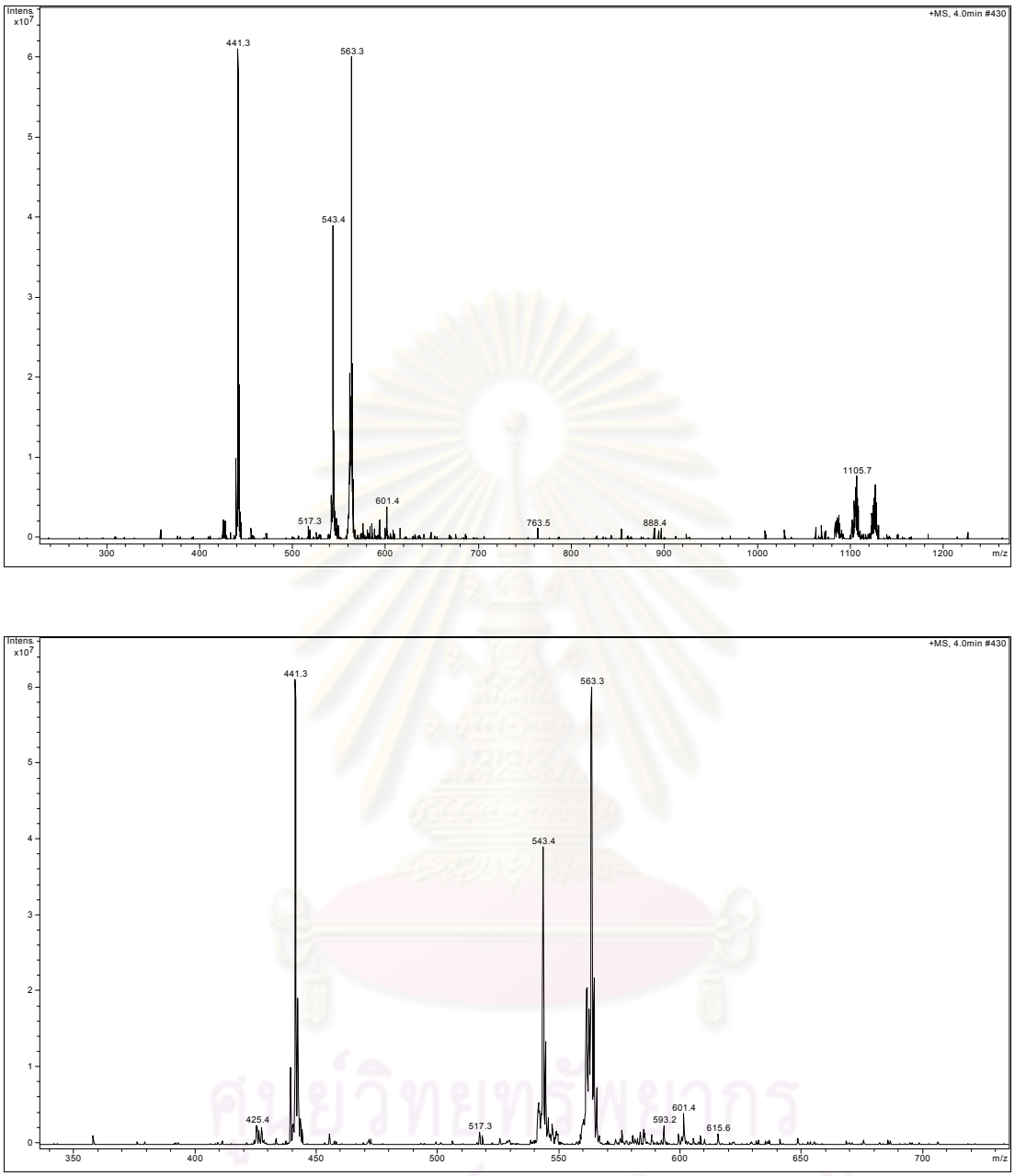


Figure 5.4 Mass spectrum of Compound 1

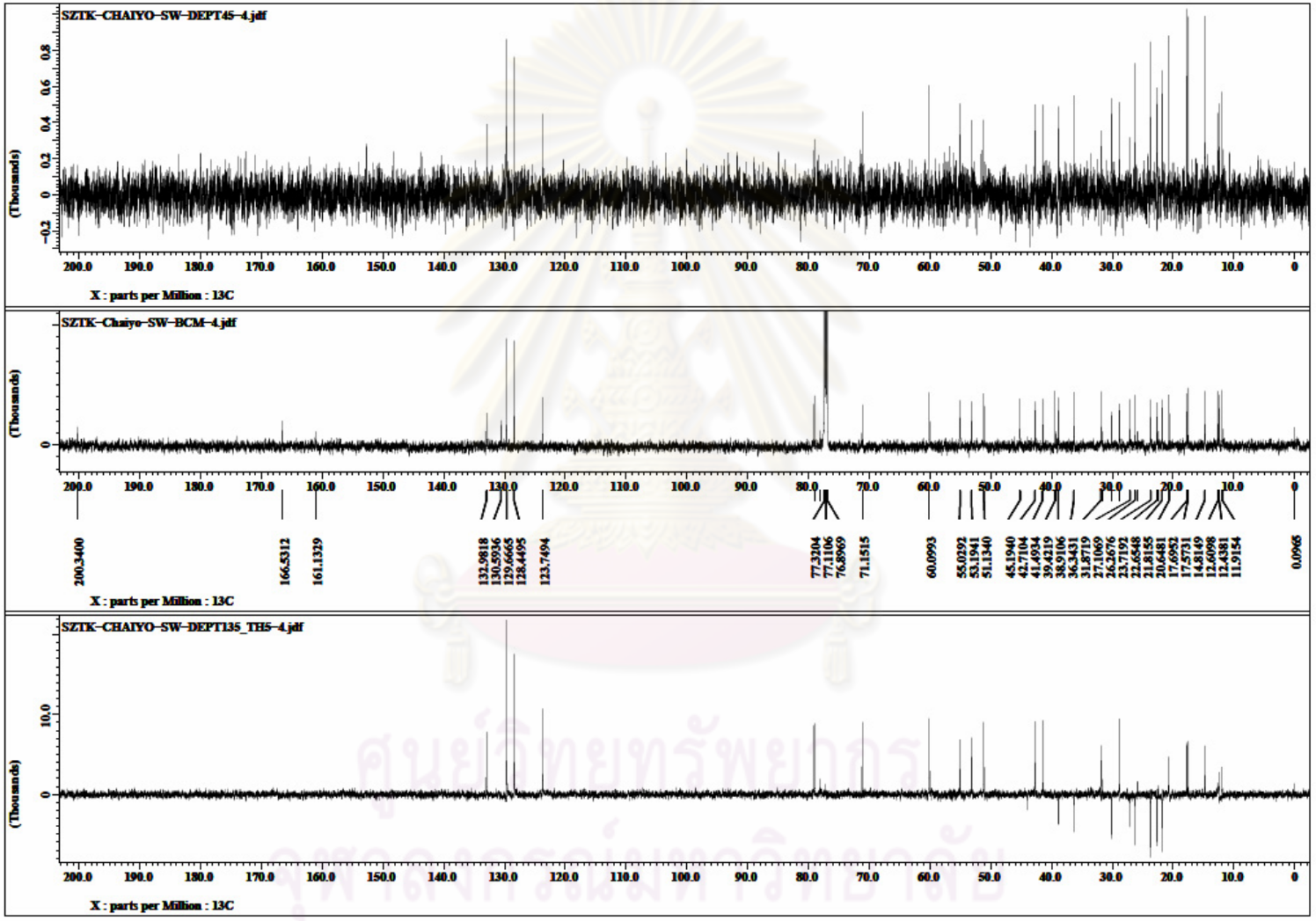


Figure 5.5 DEPT 45 and 135 spectrum of Compound 1

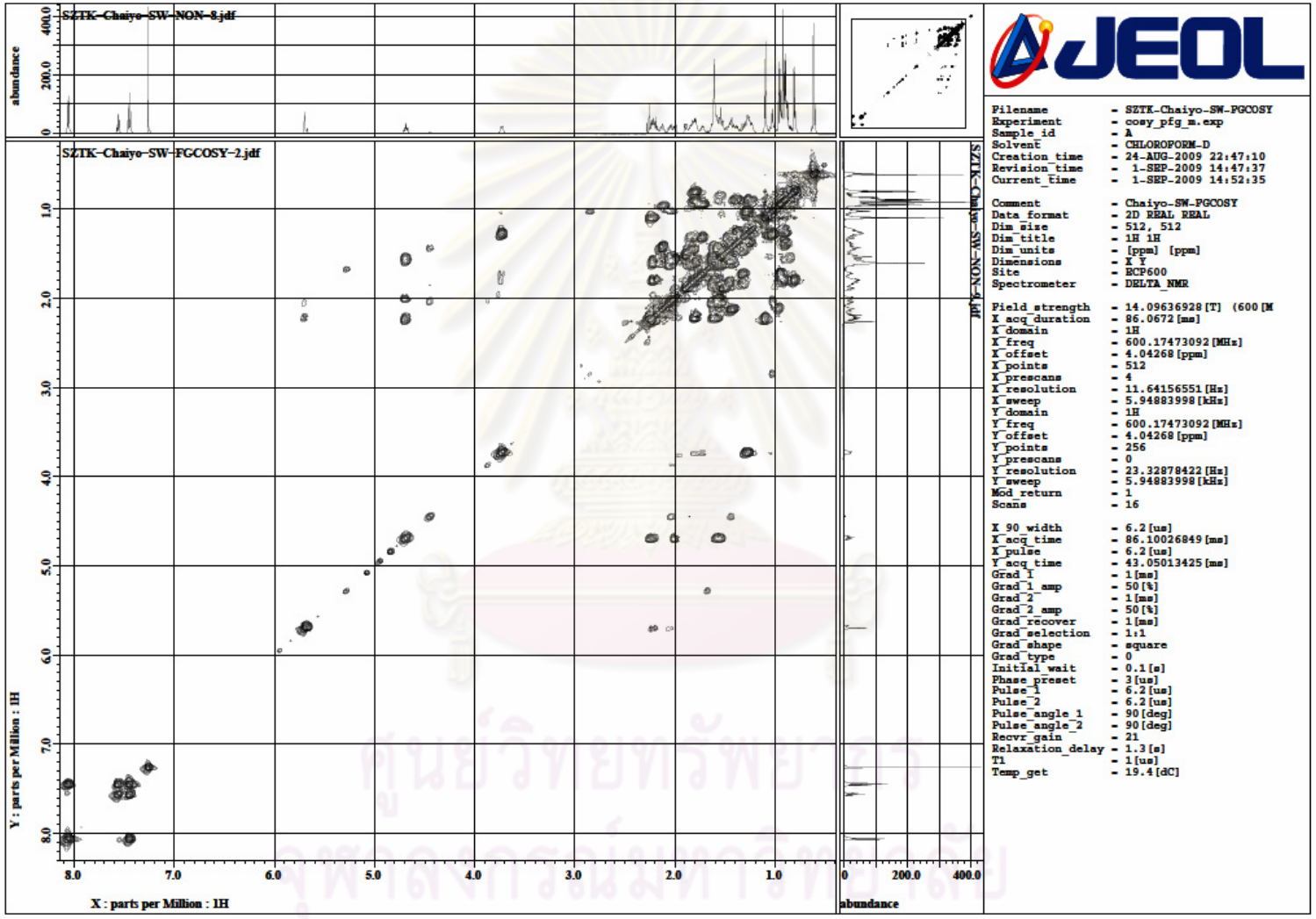


Figure 5.6 COSY spectrum of Compound 1

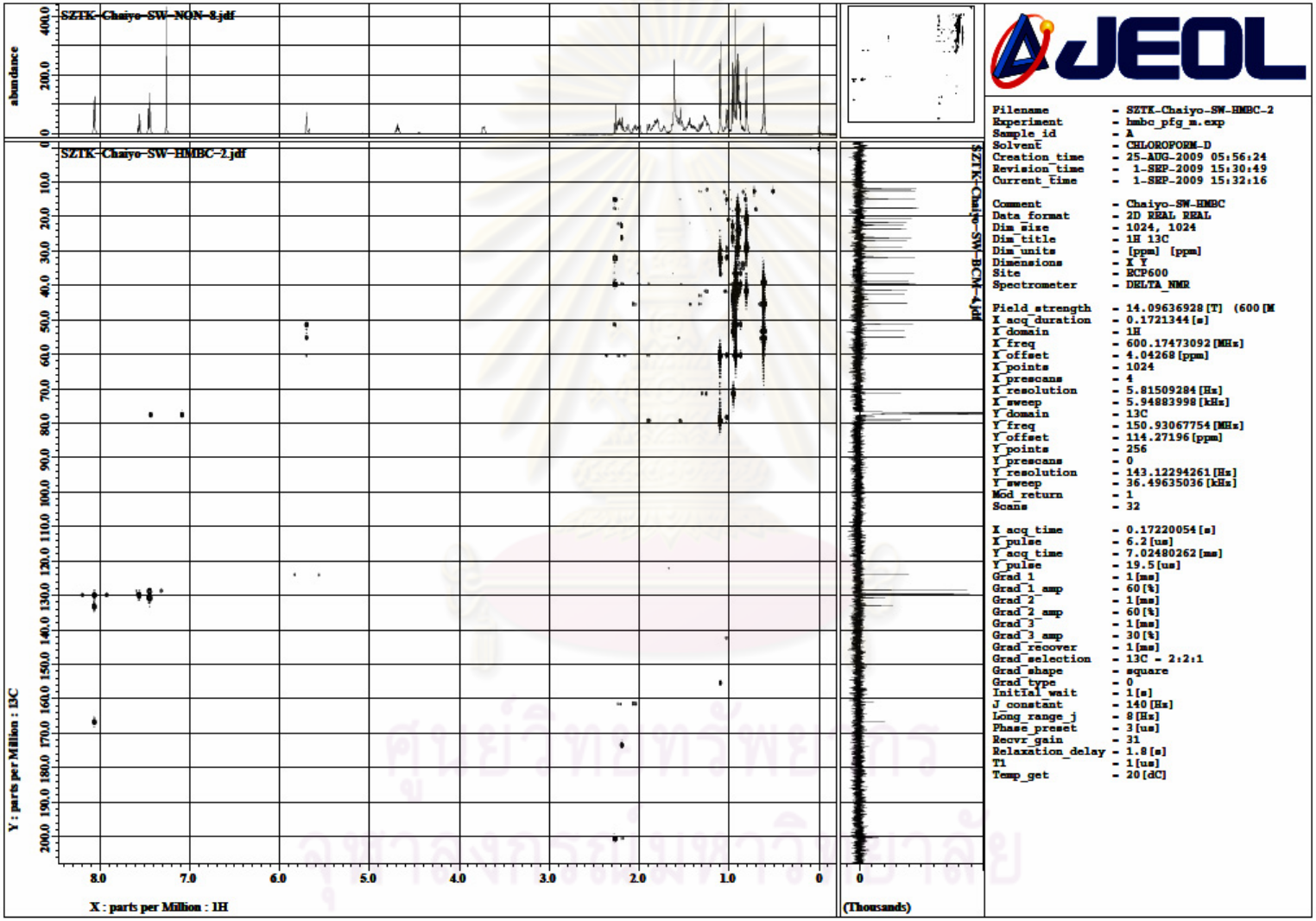


Figure 5.7 HMBC spectrum of Compound 1

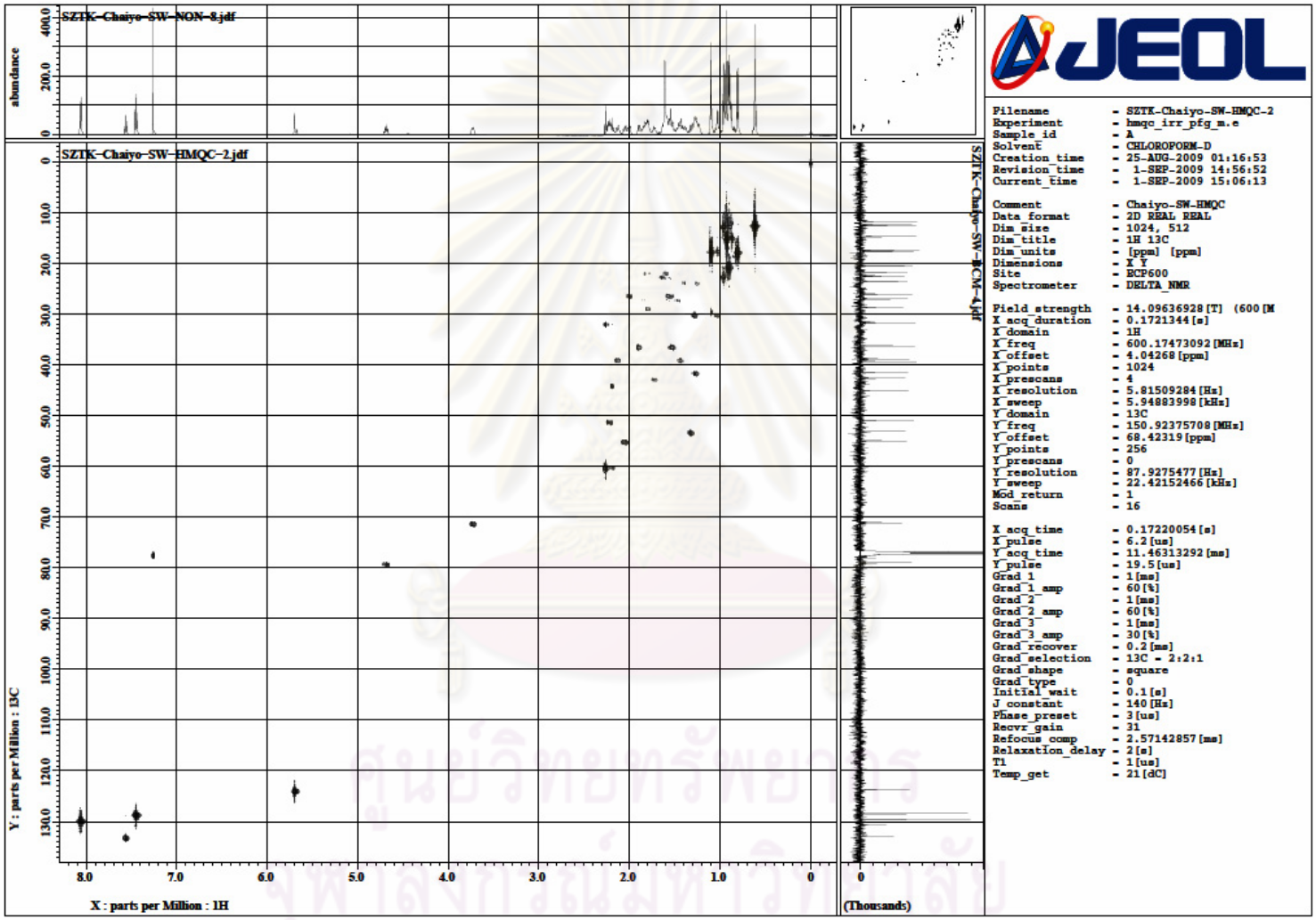


Figure 5.8 HMQC spectrum of Compound 1

Figure 5.9 IR spectrum of Compound 2

69

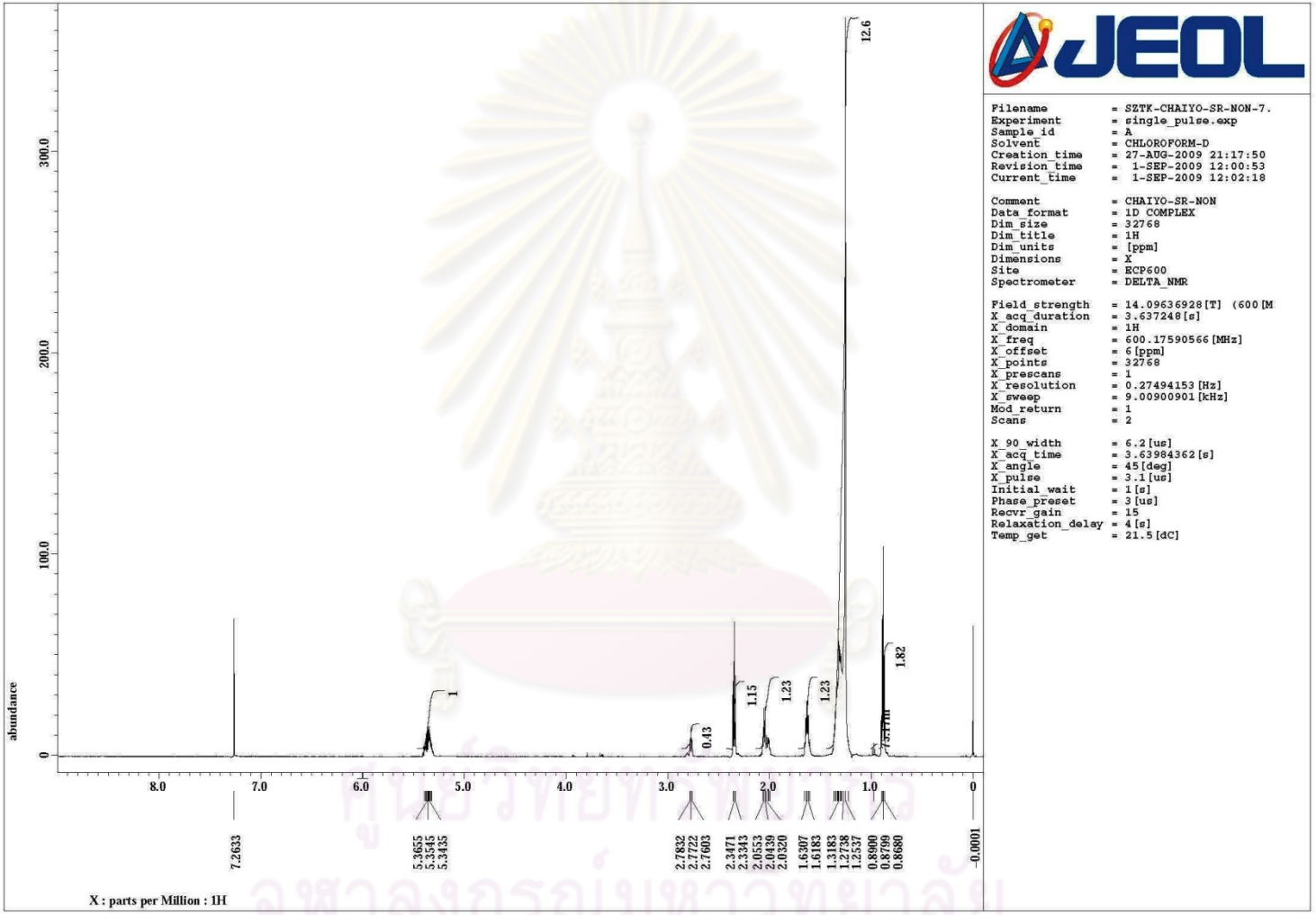


Figure 5.10 ^{1}H-NMR of Compound 2

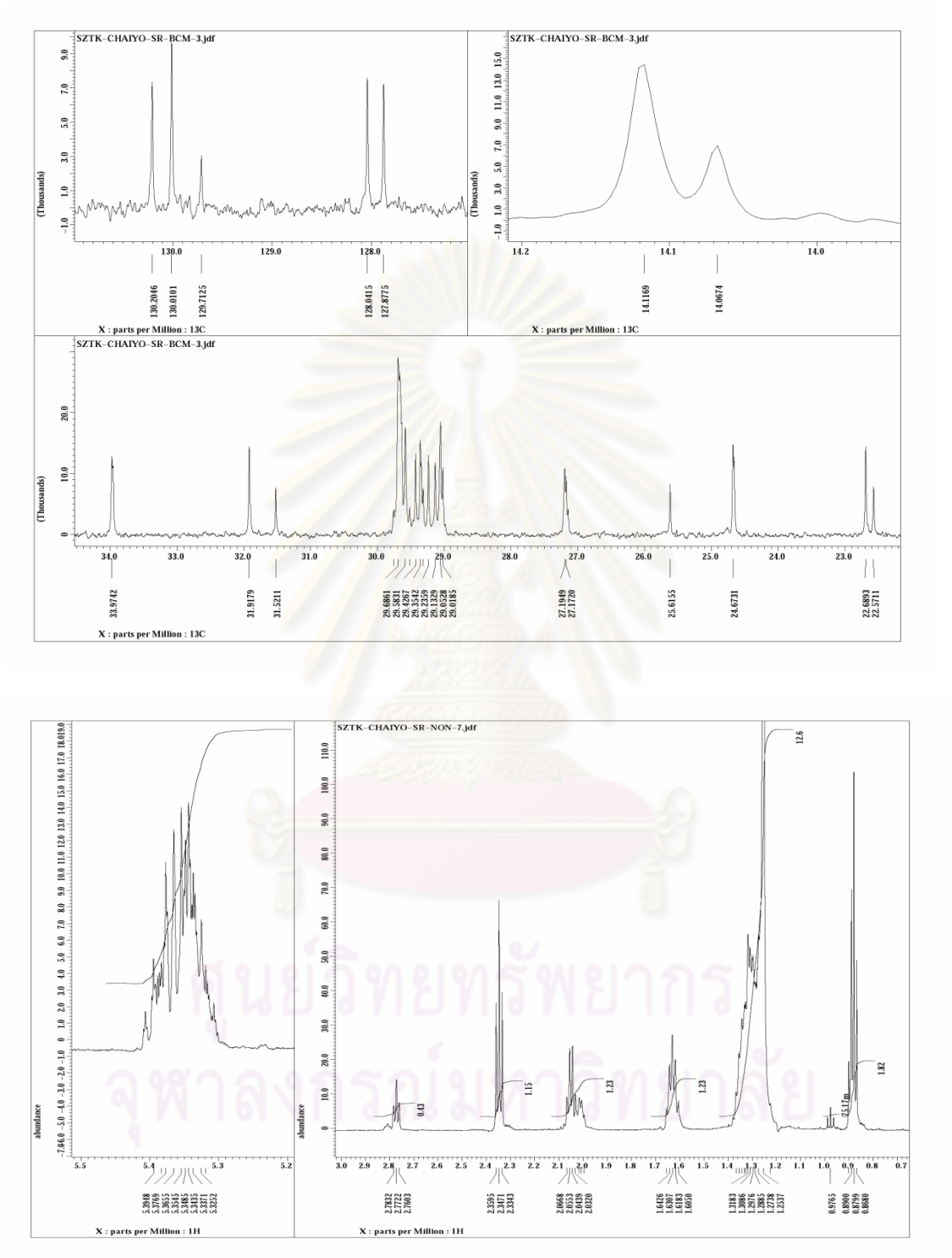


Figure 5.10 ^{1}H-NMR of Compound 2 (expand)

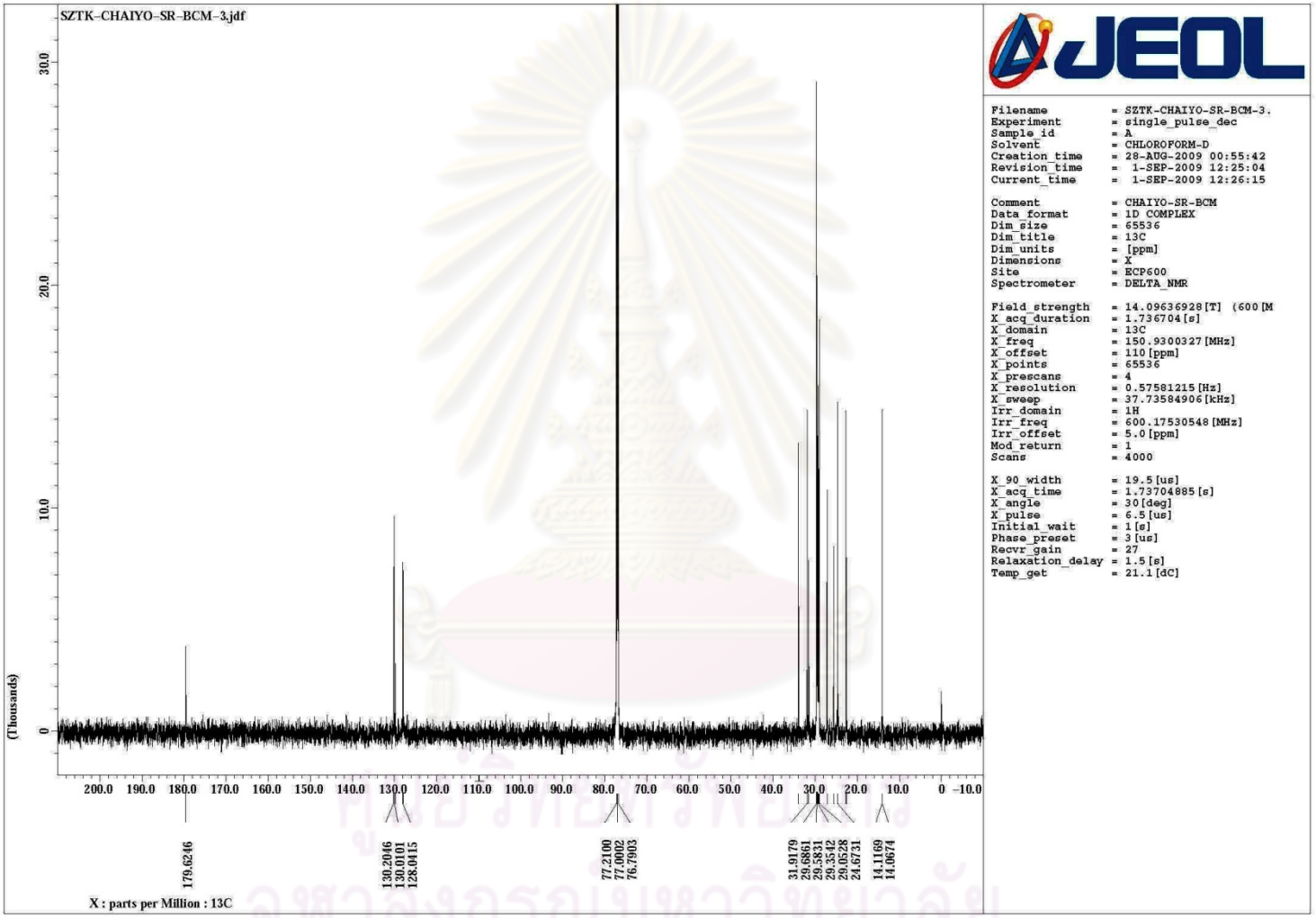


Figure 5.11 ^{13}C-NMR of Compound 2

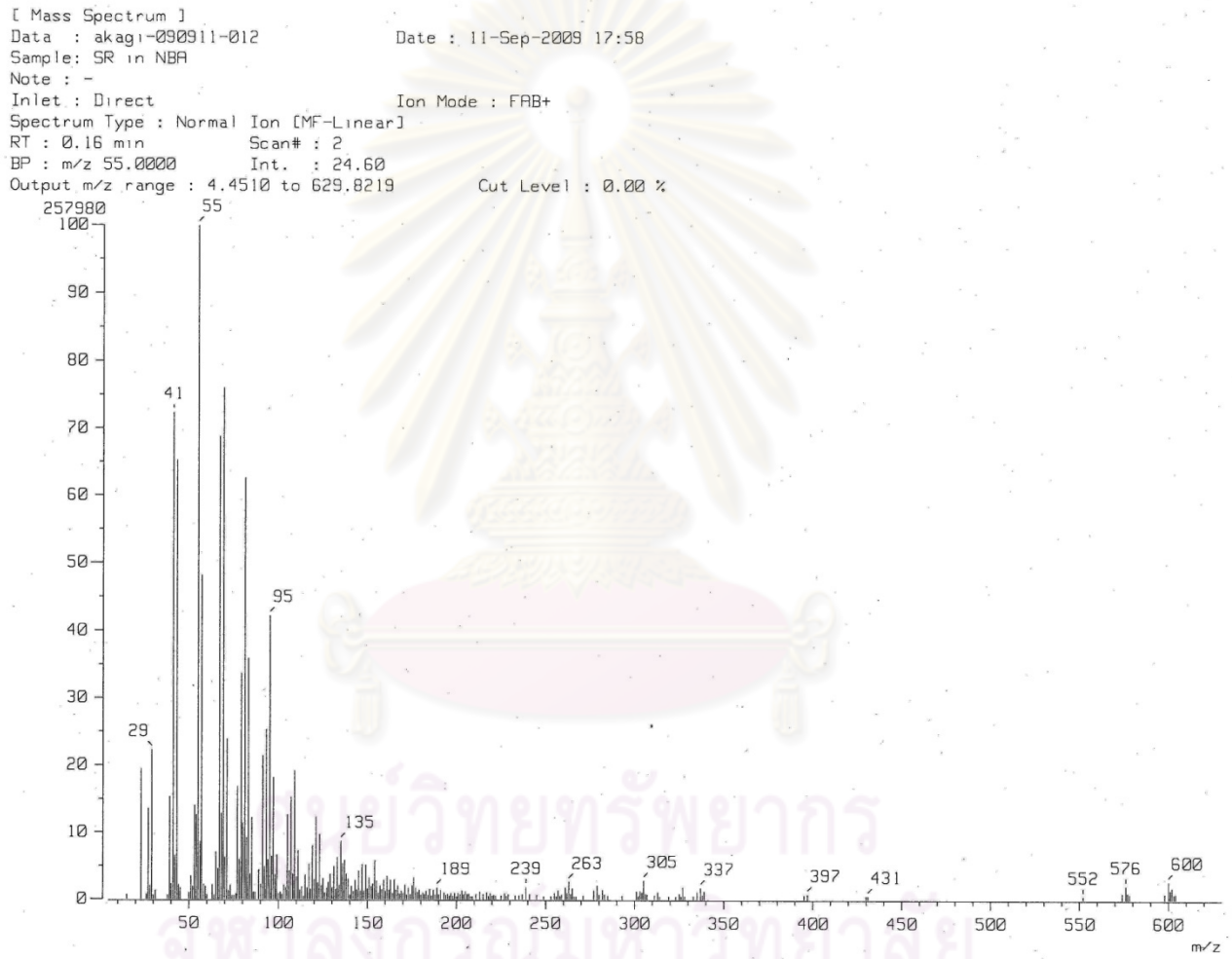


Figure 5.12 Mass spectrum of Compound 2

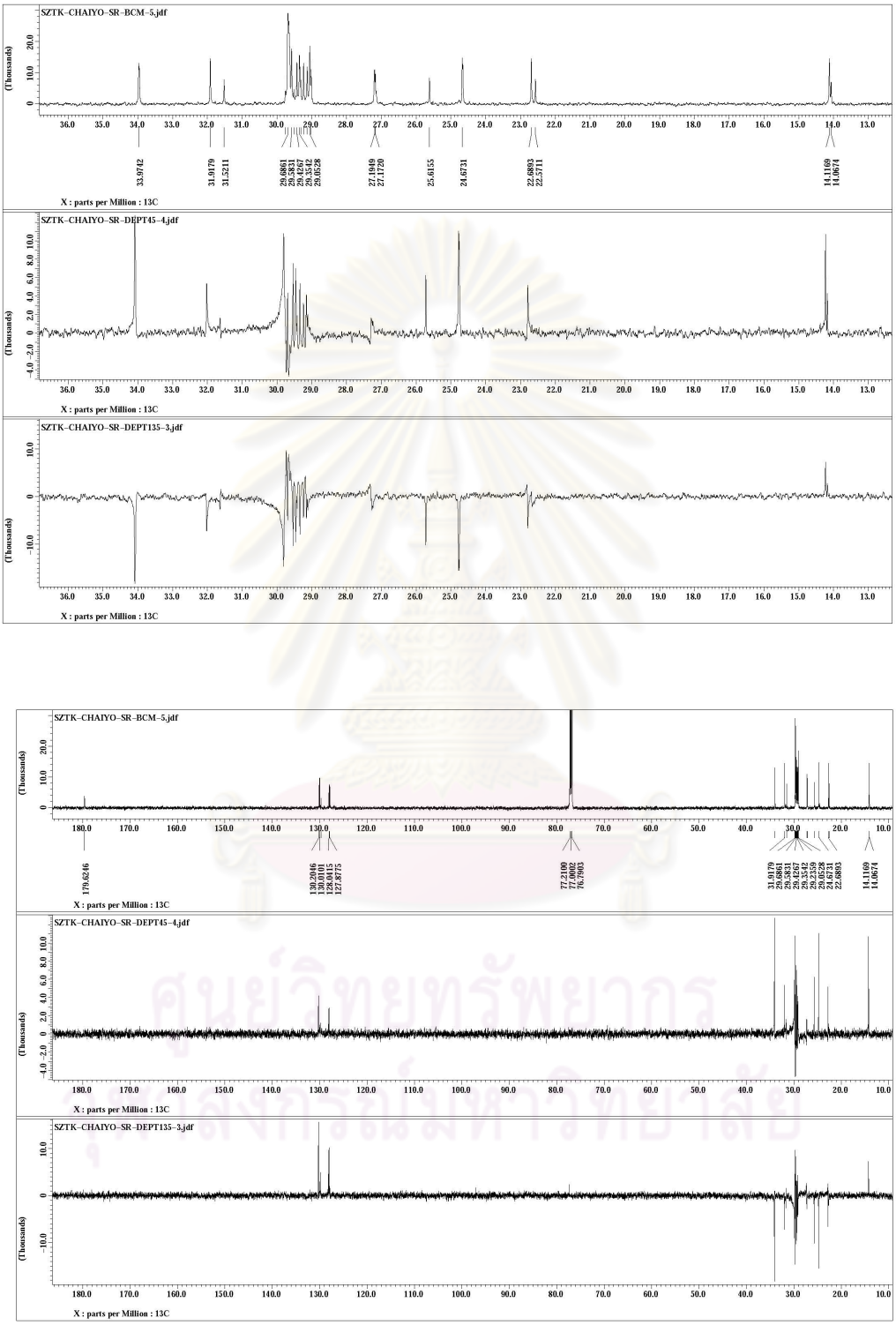


Figure 5.13 DEPT 45 and 135 spectrum of Compound 2

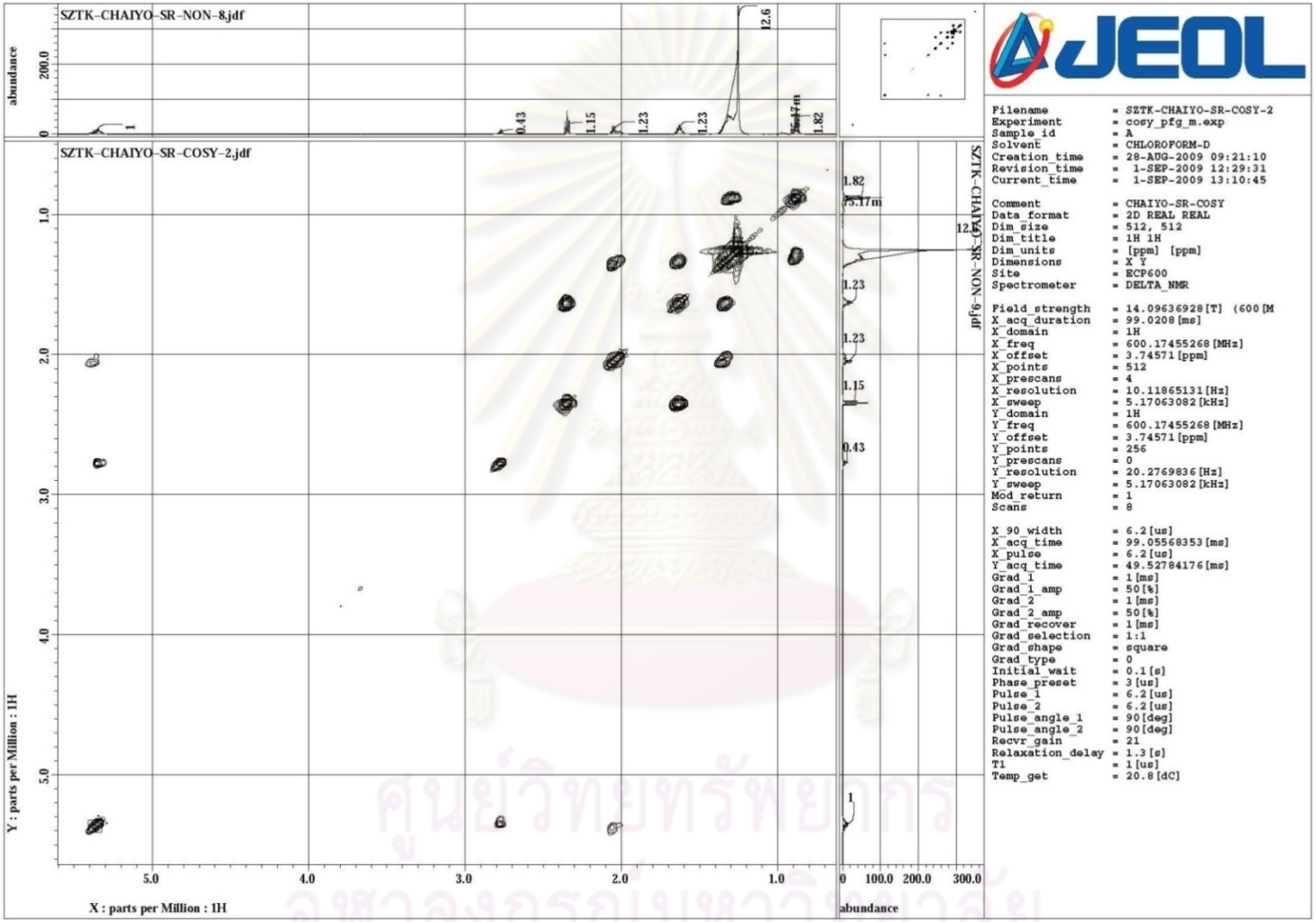


Figure 5.14 COSY spectrum of Compound 2

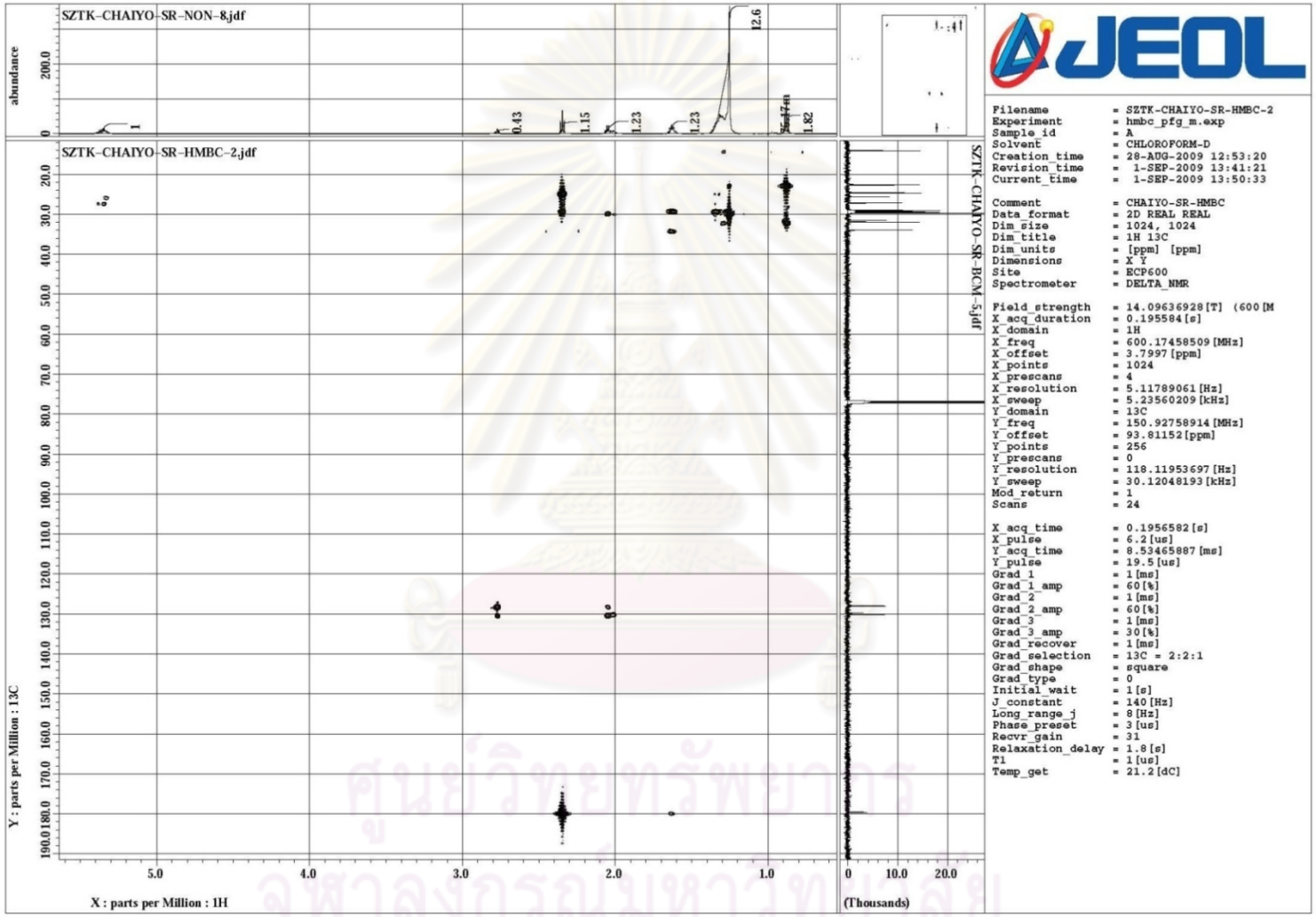


Figure 5.15 HMBC spectrum of Compound 2

Figure 5.16 HMQC spectrum of Compound 2

VITA

Miss Watinee Chanmee was born on June 21, 1984 in Nakhonratchasima, Thailand. She graduated with a Bachelor of Science Degree in Chemistry from Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand in 2007. She completed her Master of Science Degree in Organic Chemistry, Faculty of Science, Chulalongkorn University, Thailand in 2009.