CHAPTER I

INTRODUCTION

The major challenge for the world in the next millennium will be to feed itself without destroying the environment. If the forecast is right, the world population will be more than double in the next 50 years, leading to a global shortage of food, and the need to turn vast areas of wild lands over to agriculture[1]. This challenges researchers to improve the efficiency of agrochemical and get higher yields in food production. The demand will therefore be more intensive agriculture and more crop protection, rather than less embracing genetically modified crops, biological and behavioral control, and effective and environmental friendly pesticides. One important source of new chemicals for crop protection is nature. The world's biodiversity provides a seemingly inexhaustible supply of new and biodegradable compounds as potential weapon for farmer in the struggle to combat pests, weeds and diseases. Plants are a rich source and produce many potential compounds as agrochemicals (crop protection, insecticides, herbicides, fungicides and so on).

For several decades, agrochemicals have been used in agriculture. Although agrochemicals themselves have induced changes in agriculture and ecosystems with negative consequences, such as pest resistance, the destruction of beneficial organisms, chemical residues in food and water. Agrochemicals, however, are still regarded as important for securing sufficient agricultural production and increasing crop yields.

Thailand is an agricultural country. The country has approximately 513,000 square kilometers of land of which 40.9 percent is cultivable. Field crops, which accounted for 50 percent of agricultural output in 1993, increased at an annual average rate of 8 percent between 1961 and 1993. Agricultural production was still dominated by seven major crops: rice, sugar cane, mung beans, tapioca, rubber, maize and tobacco leaves, most of which were grown primarily for export. Therefore, agrochemicals used for high-value crops will continue to increase. Between 1994-1998, 38.6 percent of the imported chemicals were for agricultural sectors[2]. These agrochemicals were herbicides, insecticides, nematocides, fungicides and chemicals that modifies or controls one or more specific physiological processes within a plant called plant growth regulator (PGR).

Plant growth regulators are classified as pesticides, although their functions are very different. Pesticides essentially protect crops and prevent them from blight caused by diseases, insects and other factors. Plant growth regulators are applied on crops to increase yield and improve quality, thereby meeting commercial demand and quality standards. They also regulate the dormancy state of seed and buds, control the ratio of female to male flowers, increase the ability of plant to withstand cold, dry condition, and promote blossoming. Other functions of plant growth regulators are to prolong the time fruit stays on plants, make the plant shorter and stronger, prevent the plant from falling to the ground, prolong storage duration, and ease harvesting. In short, plant growth regulators are used to control and enhance specific chemical processes in plant to meet the demands of human consumption.

1.1 Plant growth regulator

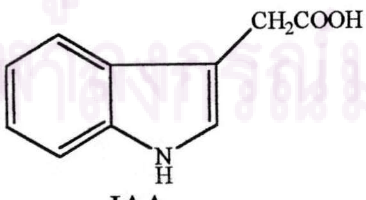
A plant growth regulator is an organic compound, either synthetic compounds e.g. indole-3-butyric acid (IBA), 1-naphthylacetic acid (NAA) or natural compounds that were extracted from plant tissue e.g. abscisic acid (ABA), cucurbic acid. A plant growth regulator is defined by the Environmental Protection Agency as "any substance or mixture of substances intended, through physiological action, to accelerate or retard the rate of growth or maturation, or otherwise alter the behavior of plants or their produce"[3]. These chemicals act on plant processes at very low concentrations. They are often produced at one location and transported to another where they exert their influence; however, they may also act on the same tissue in which they are produced.

Growth regulators play a very large part in the development of plants. There are classifiable into five groups of plant growth regulating compounds: auxins play a role in stem elongation and apical dominance, gibberellins are important in elongation, bolting and flowering, ethylene play a role in seed and bud dormancy, induction of roots, flowering, and stem elongation, cytokinin is known to influence cell division, cell and organ enlargement, and the delay of senescence in flowers, vegetables and fruit, and plant growth inhibitors such as abscisic acid (ABA). For the most part, each group contains both natural and synthetic substances.

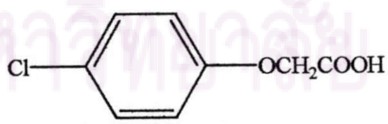
1.1.1 Auxins

Auxins were the first group of growth regulators to be discovered in the late 1800's by Charles Darwin and Ciesielski[4]. They reasoned that a signal of some sort was being transported from the plant tip resulting in curvature some distances below the tip. Later it was discovered that auxins produced in the growing tips were the signal responsible for this curvature. Today they have a wide variety of effects on plants and the effects change with concentration, the chemical form present, the presence of other growth regulators and even the growth stage of the plant. One of the most well known uses of auxins is for the rooting of cutting for plant propagation. Shoot tips of many plant species when dipped or coated with small amounts of auxins, roots develop more quickly and in higher numbers. Many herbicides are also synthetic auxins. When applied at higher (but still relatively low) concentration cause abnormal leaves curling and eventually plant death. Auxins have also been used by horticulturist in the development of parthenocarpic fruit. This is the production of fruit without fertilization which results in seedless fruit.

The natural and synthetic compounds which are classified in auxins group such as indole-3-acetic acid (IAA) and 4-chlorophenoxyacetic acid (4-CPA).



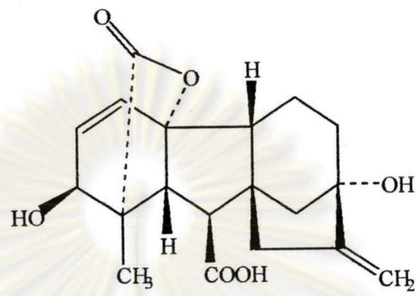
IAA



4-CPA

1.1.2 Gibberellins

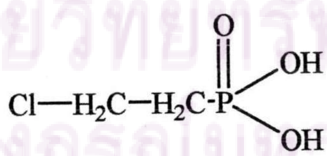
Gibberellins are a large group of related chemical compounds (over 80 have been identified) with a wide range of effects. Probably the most conspicuous and well-known effect of gibberellins is stem elongation. Applications to genetically dwarf plants known to greatly increase their growth to the point where they actually appear normal. They have little effect when applied to normal plant. Related to this stem elongation effect are the influence gibberellins having on bolting and flowering. They will often cause plants to bolt and flower even when the days are short. Based on these and other observations, researchers believe that gibberellins play a major role in controlling stem elongation in plants. In addition they are also involved in flowering and fruit development.



Gibberellic acid (GA3)

1.1.3 Ethylene and ethylene releasing compounds

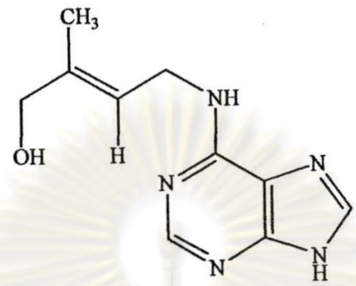
Ethylene is a very simple molecule and is produced during many combustion reactions. Fruit ripening is also a major physiological effect from ethylene. Addition of ethylene gas to fruit will accelerate ripening. Similarly if ethylene is removed, fruits will remain unripened. Fruit growers will take advantage of this fact to control the ripening of fruit during transport to the markets. Over-ripened fruit produced large amounts of ethylene and if kept it nearby other fruits will cause them to ripen and rot quickly. Ethephon (2-chloroethylphosphonic acid) is a synthetic compound that can releases ethylene.



Ethephon

1.1.4 Cytokinins

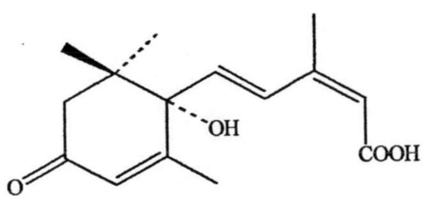
Cytokinins were discovered by scientists looking for ways to stimulate plant cell division. They are also involved in cell and organ enlargement, and delay senescence in flowers, vegetables and fruits. They may also play a role along with auxins in regulating apical dominance. It is commonly found in many plants that when the terminal shoot is removed, lateral buds will often develop and elongate. This is believed to be due to the loss of auxins from the apical meristem. In a similar fashion, artificially applied cytokinins will often stimulate dormant lateral buds to develop even in the presence of an intact terminal bud. This has led researchers to conclude that it is a balance of cytokinins and auxins which control apical dominance. Cytokinins promote cell division and lateral bud development, while auxins inhibit it. Zeatin is a natural compound from plant which was classified in this group.



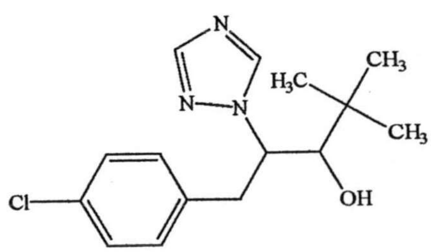
Zeatin

1.1.5 Plant growth inhibitors

Natural occurring plant growth inhibitors, which retard such physiological processes as root and stem elongation, seed germination, bud opening and so on, endogenously regulate plant development and differentiation in cooperation with auxins, gibberellins and cytokinins. The inhibitors are also excreted from some plants into the environment to retard the germination and growth of other plants directly or indirectly. One of the most active inhibitors isolated from plants is abscisic acid, which strongly inhibits the growth of young shoots, leaves, stem and roots, and promotes the abscission of plant organs, dormancy and senescence. It is widely distributed in dicotyledons and monocotyledons. Many synthetic plant growth inhibitors frequently have a haloaromatic group as a partial structure. For example, (*E*)-1-(4-chlorophenyl)-4,4-dimethyl–2-[(1, 2, 4-triazol)-1-yl]-1-pentan-3-ol, paclobutrazole, significantly reduced growth of root zone of young greenhouse-grown tissue-culture-propagated Gala apple trees[5].



Abscisic acid



Paclobutrazole

The search for more cost-effective, efficacious, selective and environmentally safe plant growth regulators has led to new emphasis on several underutilized plant growth regulator discovery strategies. These include utilization of natural products from plants. Natural products offer a huge number of chemical structure and, since many secondary metabolite compounds are presumed to have a role in interspecies interaction, many have potential as leads for new plant growth regulators.

From the result of preliminary screening test of 12 species of plants, the roots of *Tylophora indica* (Burm.f.) Merr. showed the best results of seedling growth activity against *Brassica pekinensis* Jusl. Hence, *T. indica* Merr. was selected for further investigation.

1.2 Botanical Aspect and Description[6,7,8]

Tylophora indica (Burm.f.) Merr. or *Tylophora asthmatica* Wight. belongs to the family 'Asclepiadaceae', which have 250 genera and over 2,000 species. It is one of 60 species of genera *Tylophora*, occurring in tropical and sub-tropical regions of the world. In Thailand, it has been known as 'Knanthulee' (คันธุลี), 'Thaao phan raak' (ท้าวพันราก), 'Khun phuum' (ขุนพูม) (Na Khon Phanom), 'Thao nang' (เถาหนัง) (Surat Thani), 'Nuai sai duean' (หน่วยไส้เดือน) (Chumphon)

T. indica Merr. is a herbaceous climber, small, slender, much branched, under shrub, 30-90 cm high, dark copper colored.

Leaves : 5-8 cm long, opposite, entire, ovate, roundish, acuminate, cordate at the base, glabrous above and downy below; odor disagreeable when bruised and test nauseous.

Root : twining, fleshy, whitish each being 10-13 cm long of a pale-white or brown color slender, brittle and wiry; rootstock 2.5-5 cm thick; root bark wrinkled, longitudinally fissured, marked with scars of fallen rootlets.

Flowers: minute in 2-3 flowered fascicles.

Seeds : broadly ovoid, flat.

T. indica Merr. has recently been included as one of the important drug from natural source for the treatment of respiratory diseases. It has been traditionally utilized as a folk remedy in certain region of India, not only in the treatment of bronchial asthma, but also for bronchitis, rheumatism, and dermatitis. The leaves and roots portions of this plant are medically used.

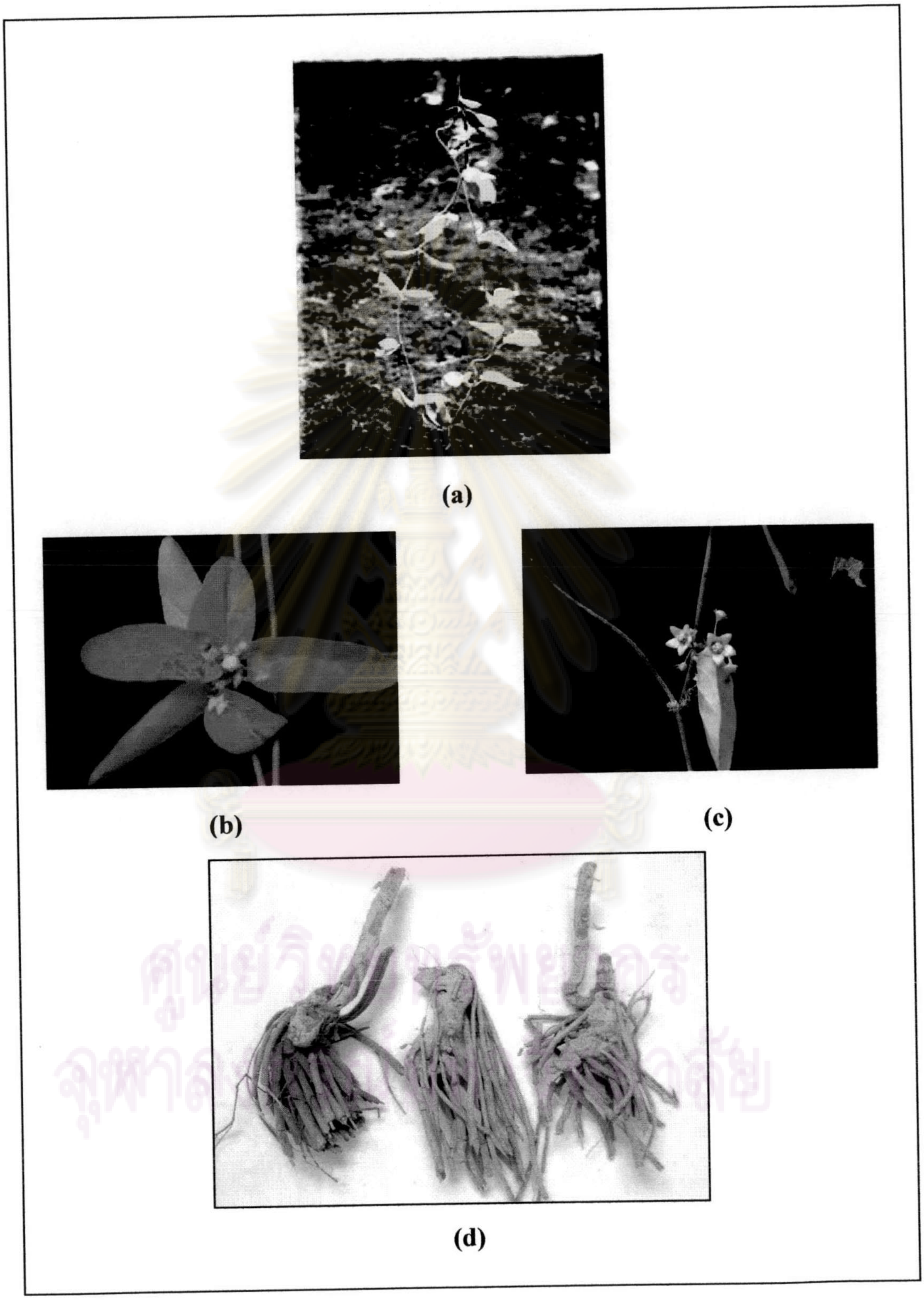


Figure 1.1 Stems (a), leaves (b), flowers (c), and roots (d) of *T. indica* Merr.

1.3 A chemical constituent studies on *T. indica* Merr.

Literature surveys of chemical constituents from *T. indica* Merr. revealed that there have been a variety of isolated organic substances (Table 1.1). The structure of some isolated compounds are shown in Figure 1.2-1.7

Table 1.1 Chemical constituents of *T. indica* Merr.

Type	Isolated compound	Part of plant	Reference
Indolizidine alkaloid	(+)-septicine (+)-isotylocrebrine	leaf	9
	(+)-14-hydroxy- isotylocrebrine 4,6-desdimethyl- isotylocrebrine tyloindicine A-E tylophorine 4-methoxy-14-hydroxytylophorine 6-demethyltylophorine 5-hydroxy-O-methyltylophorinidine tylophorinidine	aerial part	10
	desmethyltylophorine desmethyltylophorinine tylophorinine	whole plant	11
	tyloindicine F-J	aerial part	12
	tylophorinicine	root	13
Quinoline alkaloid	γ-fagarine skimmianine	root	14
Triterpenoid	α-amyrin β-amyrin	leaf	15
Steroid	campesterol β-sitosterol stigmasterol		
Flavonoid	kaempferol quercetin	whole plant	16
Other compounds	tyloindane	aerial part	12

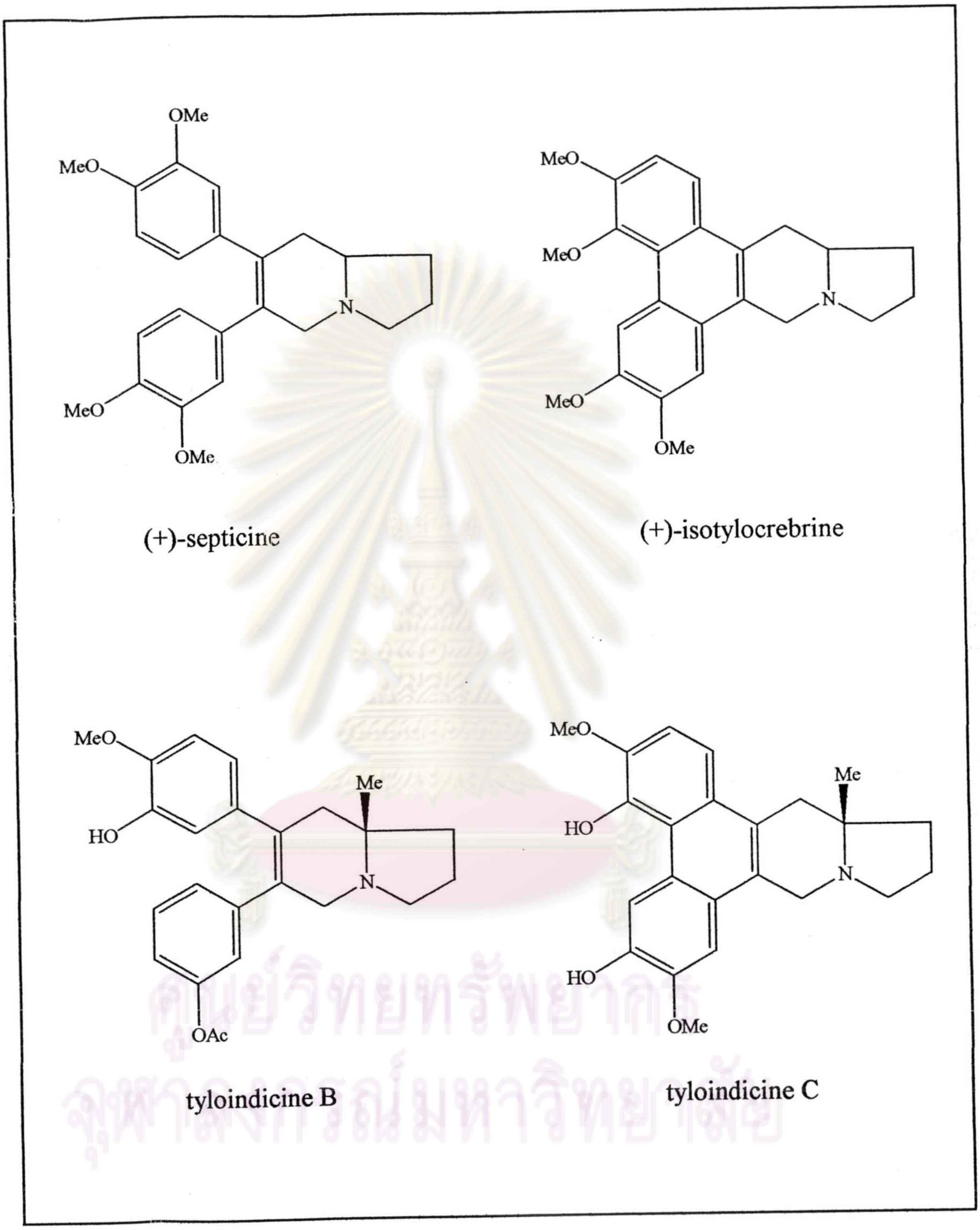
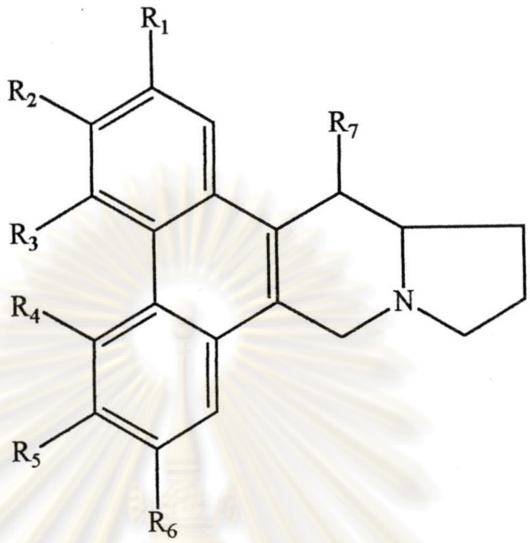
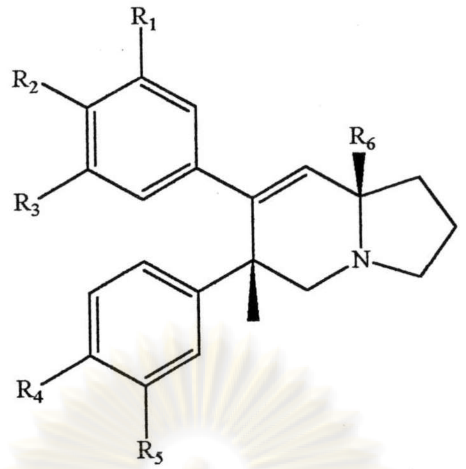


Figure 1.2 Indolizidine alkaloids from *T. indica* Merr.

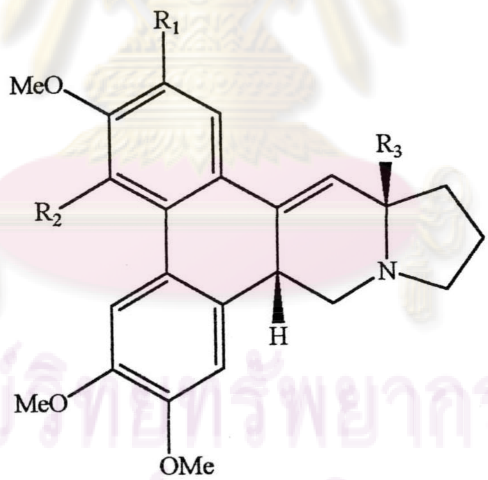


	R_1	R_2	R_3	R_4	R_5	R_6	R_7
(+)-14-hydroxyisotylocrebrine	H	OMe	OMe	H	OMe	OMe	OH
4,6-desdimethylisotylocrebrine	H	OMe	OH	H	OH	OMe	H
tyloindicine A	H	OMe	OMe	OMe	OMe	H	H
tyloindicine D	H	OMe	OMe	OH	OMe	OMe	H
tyloindicine E	H	OMe	H	H	OH	OMe	H
4-methoxy-14-hydroxytylophorine	OMe	OMe	OMe	H	OMe	OMe	OH
6-demethyltylophorine	OMe	OMe	H	H	OH	OMe	H
5-hydroxy-O-methyltylophorinidine	H	OMe	H	OH	OMe	OMe	OH
tylophorinidine	H	OMe	H	H	OH	OMe	OH
tylophorine	OMe	OMe	H	H	OMe	OMe	H

Figure 1.2(cont.) Indolizidine alkaloids from *T. indica* Merr.



	R1	R2	R3	R4	R5	R6
tyloindicine F	H	OMe	H	OMe	OMe	OH
tyloindicine I	OMe	OMe	OH	OMe	OMe	H
tyloindicine J	OMe	OH	H	OMe	OAc	H



	R1	R2	R3
tyloindicine G	OMe	H	OH
tyloindicine H	H	OH	H

Figure 1.2(cont.) Indolizidine alkaloids from *T. indica* Merr.

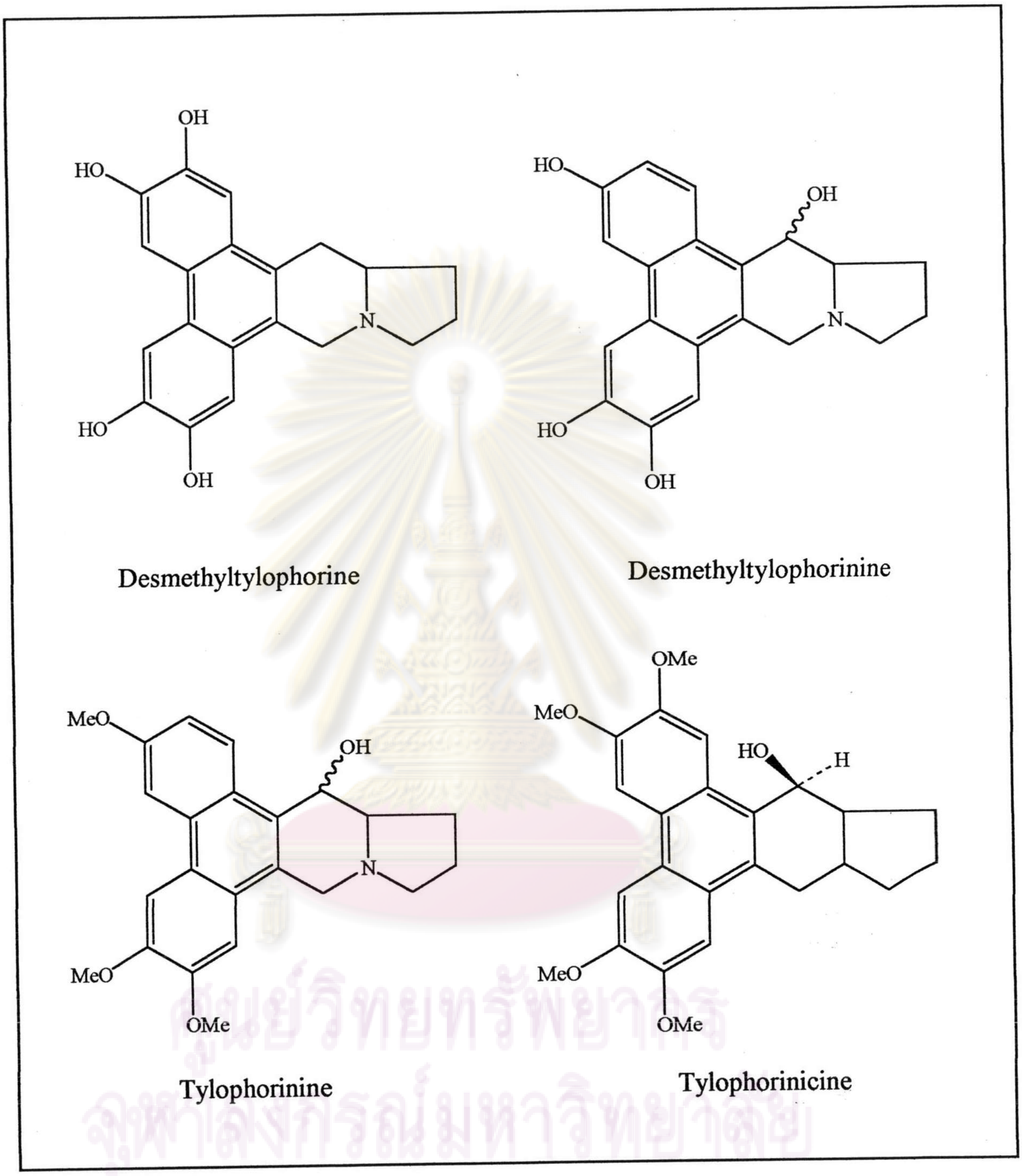
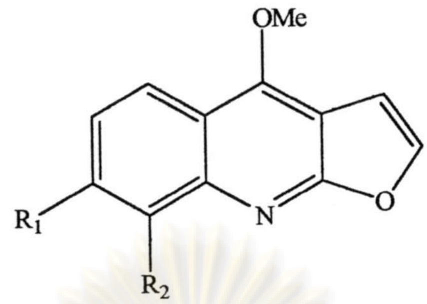
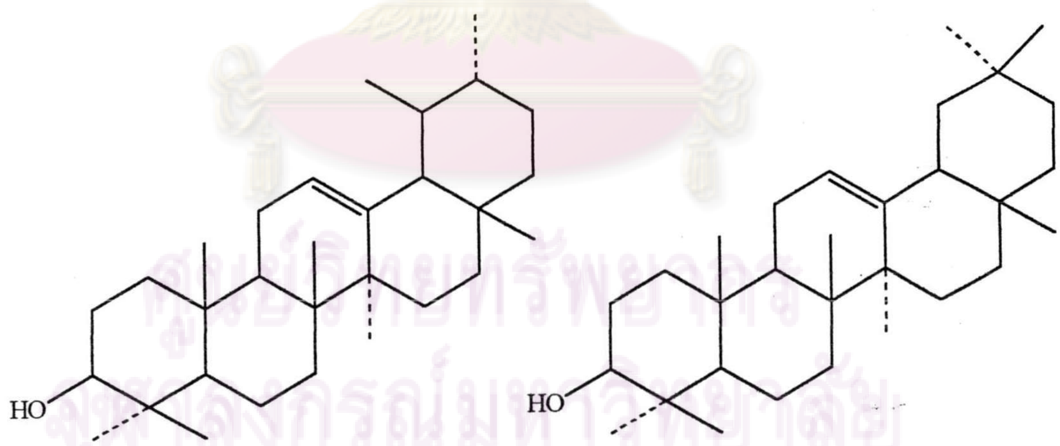


Figure 1.2(cont.) Indolizidine alkaloids from *T. indica* Merr.



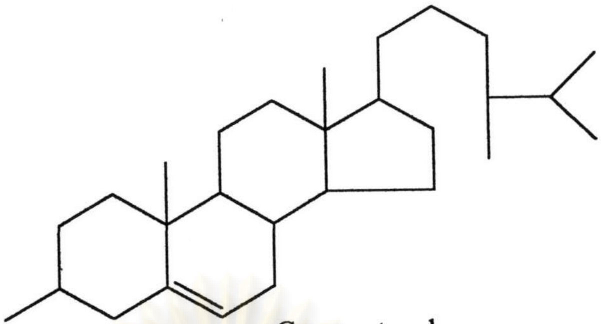
	R_1	R_2
γ-fagarine	H	OMe
skimmianine	OMe	OMe

Figure 1.3 Quinoline alkaloids from *T. indica* Merr.

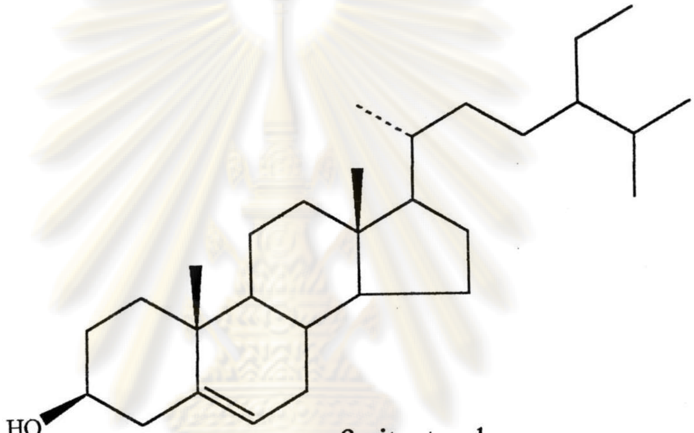


α-amyrin β-amyrin

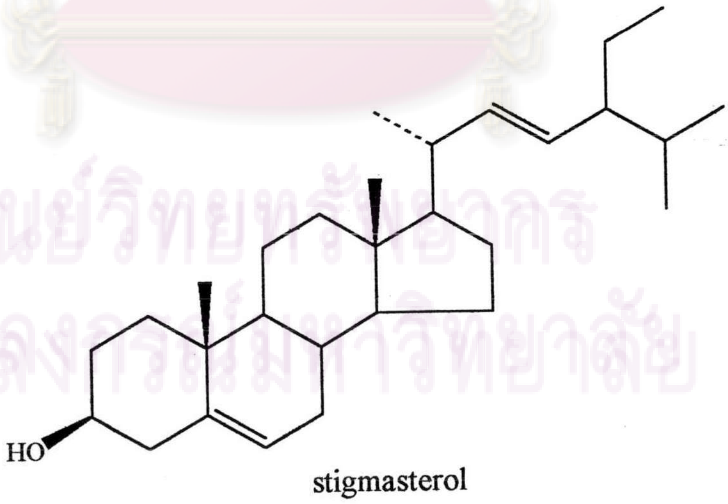
Figure 1.4 Triterpenoids from *T. indica* Merr.



Campesterol



β-sitosterol



stigmasterol

Figure 1.5 Steroids from *T. indica* Merr.

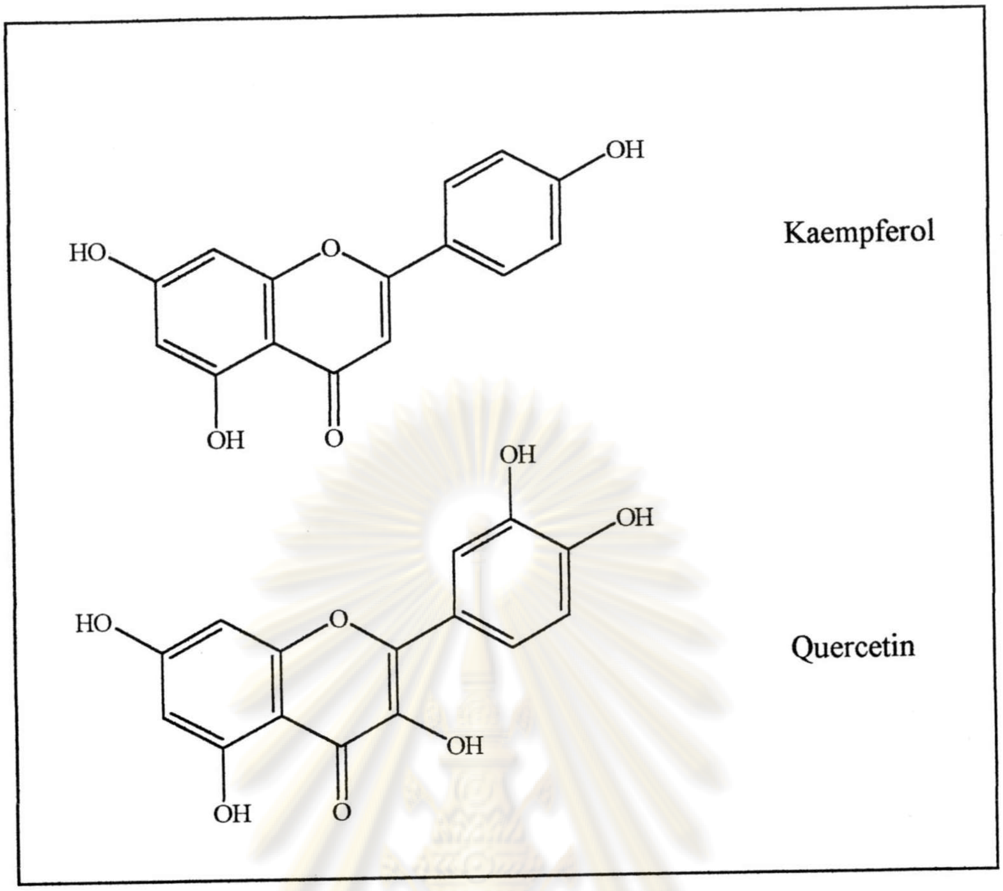


Figure 1.6 Flavonoids from *T. indica* Merr.

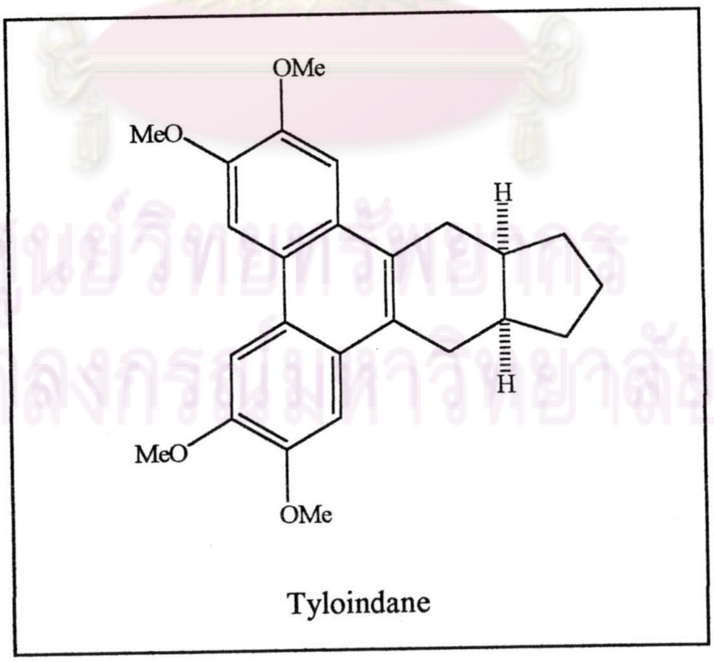


Figure 1.7 Other compound from *T. indica* Merr.

1.4 Biological Activity Studies of the Extracts of *Tylophora indica* Merr.

The biological activities of the crude extracts from *T. indica* Merr. are reported on the list in Table 1.2

Table 1.2 Biological activities of the crude extracts from *T. indica* Merr.

Part of plant	Crude extract	**Activities**	References
Aerial part	Alkaloid fraction	Antiamebic	17
	EtOH		
Leaf and stem	Alkaloid fraction	Antianaphylactic	18
		Anticholinergic	
		Glutamate-pyruvate-transaminase inhibition	
Dried bark	H_2O	Antinematodal	19
Leaf	EtOH:H_2O (1:1)	Antitumor	20
	H_2O	Antihistamine	21
		Hypotensive	20
		Leukopenic	
		Smooth Muscle Relaxant	
		Smooth Muscle Stimulant	
		Toxicity	
		Antiallergenic	22
	Hot H_2O	Antiasthmatic	23
	100% EtOH	Adrenal Hypertrophy	24
	95% EtOH	Immunomodulator	25
		Immunosuppressant	
	MeOH	Antimitogenic	26
	Alkaloid fraction	Immunomodulator	
Stem	EtOH : H_2O (1:1)	Antitumor	21
Root	EtOH : H_2O (1:1)	Cytotoxicity	27
	H_2O		

1.5 The Goal of this Research

Based on primary screening results on seedling growth of *B. pekinensis* Rupr., the roots of *T. indica* Merr. were selected for further investigation on chemical constituents which showed the highest effect. The goal of this research could be summarized as follows:

1. To extract and isolate the organic substances from the roots of *T. indica* Merr.
2. To elucidate the structural formula of the isolated substances from the active crude extract that show the promising activity.
3. To search for substances which show the plant growth regulator activity against *Brassica pekinensis* Rupr.

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