CHAPTER 1
INTRODUCTION

Cassia siamea Lamk. plant is a shrub grown only in tropical area. It is classified in the Genus Cassia Family Caesalpiniaceae. This plant is approximately 8-9 metres in height. Its leaves have a pinnate type with 7-10 pairs of leaflets and 3-4 cm. in length. The flowers are yellow and have long spike. Its pod has a flat shape and 20-30 cm. in length (Figure 1). In Thailand, it commonly found in all parts of the countries with different local names depended on the growing areas such as in central part, it is called "Khi-lek-loung" or "Khi-lek", in the north it is called "Khi-lek-ban" and in the south it is called "Ya-ha".

Cassia siamea Lamk. young leaves and flowers has been used as ingredients in several Thai cooking reserpies. Likewise several parts of this plants have been used for traditional medical practice for longtime. Its roots and barks are used as antipyretic drug (Kittikajorn, 1983). The first experimental evidence demonstrating the effect of crude ethanol extract of young leaves and flower in volunteers patients and animals were reported by Arunlakshana (1947). It was also found that crude ethanol extracts of the two parts have shown potent central nervous system depressive effect on both species. Then, the crude extract was used to treat patients who suffered with insomnia and anxiety. The author also suggested that the crude extract may act at the central nervous system since it inhibits the stimulating effect of strychnine. Since then, there have been no work done on this matter for four decades. The major ingredient was isolated from the leaves of *Cassia siamea* and its chemical structure has been evaluated as chromone derivative by Hassanali (Hassanali, 1969). It was found later by Arora (1971) that this chromone reacts with acid and

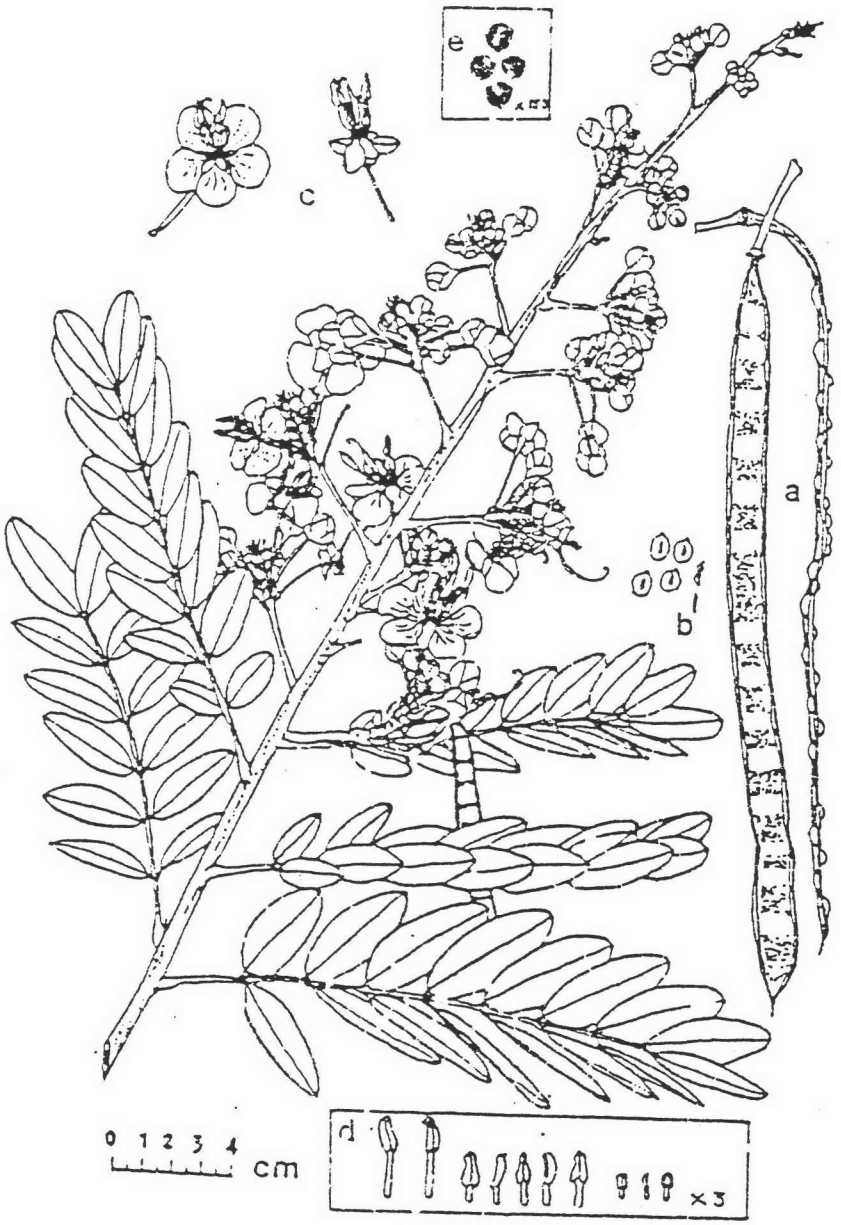


Figure 1 : Detailed drawing of several parts of*Cassia siamea* Lamk.

(Pongboonrod, 1981).

a pods b seeds c flower

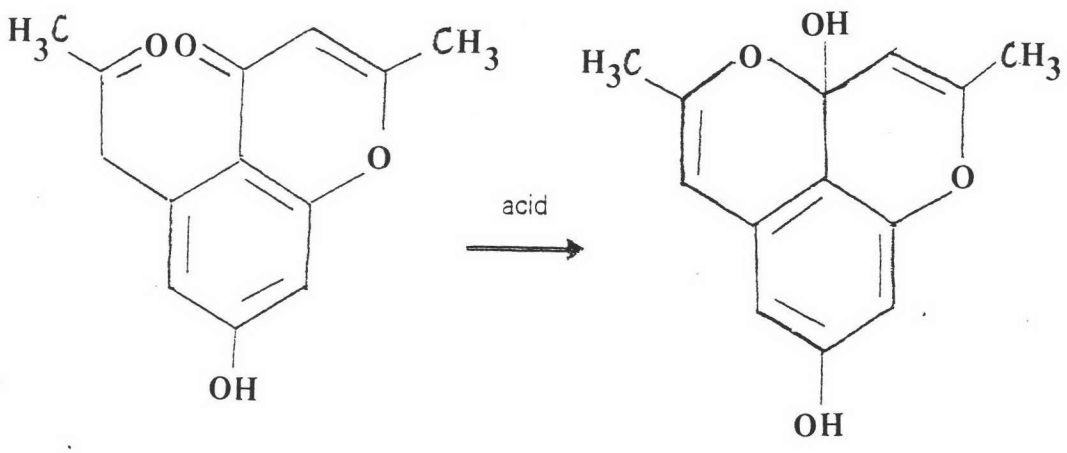
d staments e pollens under microscope

transforms to 3α,4-dihydro-3α,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene (barakol) (Figure 2).

General background of Barakol

Barakol ($C_{13}H_{12}O_4$) is a pale lemon yellow needle-shaped crystal with the melting point at 165 °C. This substance is less soluble in methanol, ethanol, and acetone, moderately soluble in chloroform and dichloromethane, and readily soluble in benzene, carbontetrachloride, ether, and water (Bycroft, 1970). In normal condition, the barakol is unstable by loosing water molecule and change to anhydrobarakol, ($C_{13}H_{10}O_3$) which is the dark green amorphous compound. However, this compound can be reconverted into barakol by dissolving in aqueous methanol. The stability of barakol is improved by reacting with concentrated hydrochloric or hydrobromic acid to a methanolic solution of barakol, giving anhydronium salt (anhydrobarakol hydrochloride) (Fig. 3,Bycroft, 1970).

Barakol has been reported to decrease locomotor activity in mice at the dose ranging 5-275 mg/kg BW. The substance has also been found to enhance the effect of apomorphine in increasing of the turning of ipsilateral substantia nigra-lesioned rat (Jantarayota, 1989). This substance was less effect since the LD_{50} was found to be 324 mg/kg BW. The author, later concluded that barakol is a dopamine agonist. The decrease of locomotor activity has been confirmed by Kaokeaw (1993) and the author also successfully prepared [I]anhydrobarakol hydrochloride from the reaction between anhydrobarakol hydrochloride and sodium iodide in the presence of oxidizing agent, chloramine-T. Two iodine atoms were found to substitute two hydrogen atoms at ortho position of hydroxyl group in the structure of anhydrobarakol hydrochloride. At



5-acetonyl-7-hydroxy-2-methylchromone

Barakol

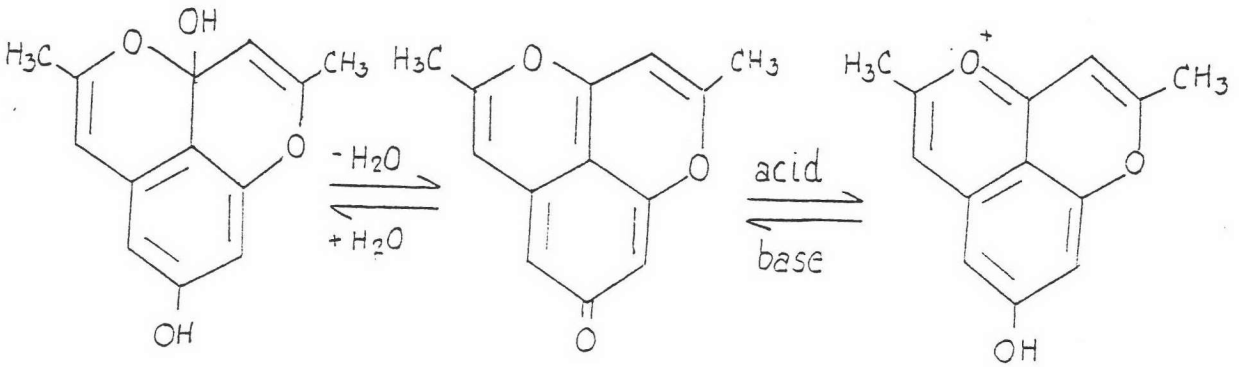
Figure 2 : Transformation reaction of 5-acetonyl-7-hydroxy-2-methylchromone to barakol

dose 60 mg/kg BW, the iodinated compound was shown to produce less depressive effect (30%) than anhydrobarakol hydrochloride (Kaokaew, 1993).

These pharmacological properties of barakol suggested that barakol may be a potential good candidate for natural sedative drug. The advantage of the medicinal plant has been widely accepted over the synthetic drug due to its less side effects. However, the employment of this substance as the drug requires more detailed studies and clinical trials to ensure its efficacy and safety. Evidences which are necessary to be revealed first is whether or not barakol induced sedative effect through binding sites in the central nervous system. What part of the central nervous system and what neural system contain those binding sites. These will be achieved by receptor autoradiography technique developed by Kuhar (Kuhar. 1975).

General Background of Autoradiography Techniques

Localization of neurotransmitter receptors by autoradiographic technique has become popular for the study of receptor distribution in the brain and other organs over 25 years. The technique is able to demonstrate the drugs exerts their effect via receptors in the brain region and neuronal pathways. It is a guide to other kinds of experiments where drugs or neurotransmitter are injected directly into brain. The usual sequence for localizing receptors by autoradiography is to label or tag the receptor of interest with a radiolabeled ligand such as iodine-125 (^{125}I),tritium (^{3}H),in an intact animal or tissue section. The radiolabeled ligand emitted rays including α-rays, γ-rays or β-rays to react with grain density on the film, and then to generate an autoradiogram which reveals the receptor bound ligand. The *in vitro* labeling approach has many advantages over the *in vivo* labeling approach. The *in vivo* receptor labeling is applied to the procedure in which receptor are labeled in intact living tissue after



Barakol Anhydrobarakol Anhydronium salt

(Anhydrobarakol hydrochloride)

Figure 3 : Conversion reaction among barakol, anhydrobarakol and anhydronium salt (Anhydrobarakol hydrochloride) (Bycroft, 1970).

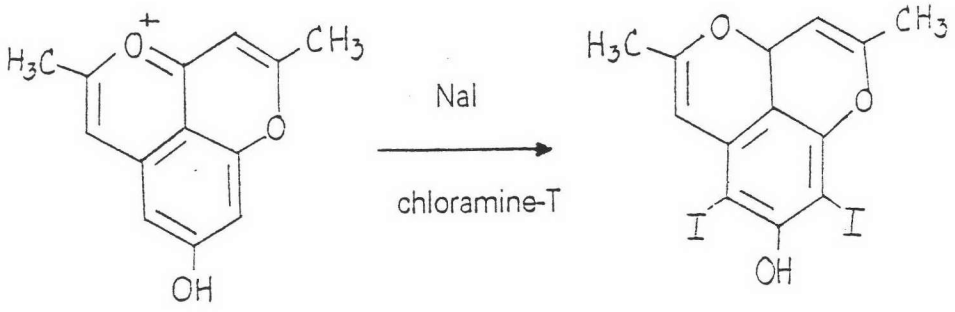


Figure 4 : Iodination reaction of anhydrobarakol hydrochloride

systemic administration of ligand or drug. The drug is carried to the brain by the blood, diffuses into the brain, and binds to the receptors. The nonbound drug is then removed from the brain and other tissues by various excretory processes. Nevertheless, *in vivo* labeling procedures have serious limitations. One is that only ligands with high affinity for receptors and cross the blood brain barrier can be used successfully (Kuhar, 1986). For the *in vitro* labeling autoradiographic procedure, receptors are labeled in slide-mounted tissue sections by incubation with radioactive ligand so that the receptors are labeled under very controlled conditions. Following the labeling, the slide-mounted tissue sections can be rinsed to remove nonreceptor-bound drug and improve the specific to nonspecific ratios. The *in vitro* labeling provides greater specificity and efficiency. Inhibitors can be added to prevent metabolism of the labeling ligand, and ligand that will not cross the blood brain barrier, or that are rapidly broken down in the blood can be used (Kuhar, 1981). It is also possible to carry out studies with human postmortem tissue. Also, *in vitro* labeling is significantly less expensive than *in vivo* labeling because an entire animal does not have to be injected with large amounts of a radioactive ligand (Kuhar 1986). Example of receptor with their specific ligands obtained from the *in vitro* receptor binding studies are listed in Table 1.

In this study, the experiments were performed *in vitro.* Receptor autoradiographic study is conducted by using [^{125}I]anhydrobarakol hydrochloride for identification and localization of barakol binding sites in rat brains. These results may be worthwhile in supporting further studies of pharmacological actions of barakol in the central nervous system and in turning a natural resource into a medicinal one.

Table 1 Examples of receptors with their specific ligands obtained from the *in vitro* receptor binding studies.

Receptor	Ligand	Reference
Thyrotropin-Releasing Hormone (TRH)	[^{3}H]MeTRH	Manaker et al 1986
Benzodiazepine	[^{3}H]flunitrazepam [^{3}H]flunitrazepam	Negro et al 1995 Yong et al 1979
D_1, D_2 and DA uptake sites	[^{3}H] SCH 23390 [^{3}H] spiperone [^{3}H] mazindol	Przedborski et al 1991
Protein Kinase C	[^{3}H] PDBu (phorbol 12,13-dibutyrate)	Horsburgh et al 1991
Cocaine	[^{125}I] RTI-55	Staley et al 1994
D_2 receptor	[^{125}I] IBZM	Brucke et al 1988
Muscarinic cholinergic	[^{3}H] pirenzepine for M_1 [^{3}H] AF-DX 116 for M_2	Kostic et al 1991
D_2 transporter	[^{3}H] WIN 35,428 [^{125}I]RTI-55	Coulter et al 1995
GABA	[^{3}H] muscimol	Penney et al 1981
Opioid	[^{3}H] GR opioid	Young et al 1979
5-HT_4	[^{3}H] GR 113808 [^{3}H] BIMU-1	Jakeman et al 1994