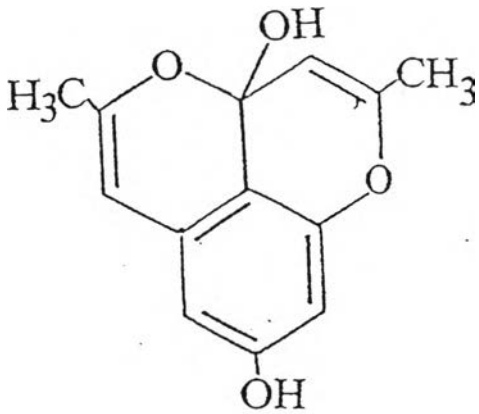
CHAPTER 2
LITERATURE REVIEW

The first experimental evidence demonstrating the effect of crude ethanol extract from the young leaves and flower of Khi-lek on the volunteer patients and the animals was reported by Arunlakshana (1949). Crude ethanol extracts from these two parts showed a potent central nervous system depressive effect on both species. Then, the crude extract was used to treat patients who suffered from insomnia and anxiety. The author also suggested that the crude extract may act on the central nervous system since it inhibits the stimulating effect of strychnine. Since then, there has been no work done on this matter for four decades.

In late 1969, an active compound was first extracted from leaves and flowers of *Cassia siamea*, Lamk (Hassanali, 1969) and the structure was characterised in 1970 (Bycroft, 1970) which was called "barakol" (3α, 4-dihydro-3α, 8-dihydroxy, 5-dimethyl-1, 4-dioxalophenalene) (Figure 2). The process of isolation was later improved by acid extraction (Chaichantipyuth, 1979 and Kaokaew, 1992) which gave a better yield (0.1-0.3%).

Chemical properties of barakol

Barakol ($C_{13}H_{12}O_4$) is a greenish-yellow needle crystal and has a melting point at 165 ^{o}C. This substance is readily soluble in methanol, ethanol, acetone, benzene, carbontetrachloride, ethyl acetate and water, moderately soluble in chloroform and dichloromethane (Hassanali, 1969). The barakol is usually extremely unstable in normal conditions when it loses a water molecule and becomes a dark green amorphous compound called anhydrobarakol. However, this substance can be easily reconverted to barakol by dissolving it in an aqueous methanol (Bycroft, 1970). However, its stability can be improved by the addition of concentrated hydrochloric or hydrobromic acid to methanolic solution of barakol, giving anhydronium salt (anhydrobarakol hydrochloride or anhydrobarakol hydrobromide). The chemical structures of barakol, anhydrobarakol



Barakol

Figure 2 Chemical structure of barakol.

and anhydronium salt have been evaluated by spectroscopic studies (Figure 3; Bycroft, 1970 and Kaokaew, 1992).

Pharmacological properties of barakol

Barakol (5-100 mg/kg, i.p.) has been reported to produce a dose-related decrease in locomotor activity in mice (Jantarayota, 1987 and Kaokaew, 1992). The sedative effect was observed at low dosage (5-10 mg/kg) but increased dosage slowed the mice further. They did not move, but they were not asleep. This report is the same as the results of Arunlukshana (1949). The toxic effect of barakol was very low because the lethal dose-50 (LD50) in mice was 302-347 mg/kg body weight (Jantarayota, 1987).

The anxiolytic effect of barakol was studied in an elevated plus maze. It showed anxiolytic effect (anti-anxiety) and hyperlocomotor activity at a low dosage of barakol (10 mg/kg, i.p.). On the other hand, the higher dosages (25, 50 mg/kg, i.p.) showed sedative effect and hypolocomotor activity in mice (Thongsaard, 1996). But in a later experiment barakol (5, 10, 15 and 20 mg/kg, i.p.) did not show an anxiolytic effect on an elevated plus maze and shock probe burying test, but the sedative effect was observed in this experiment (Fiorino, 1998).

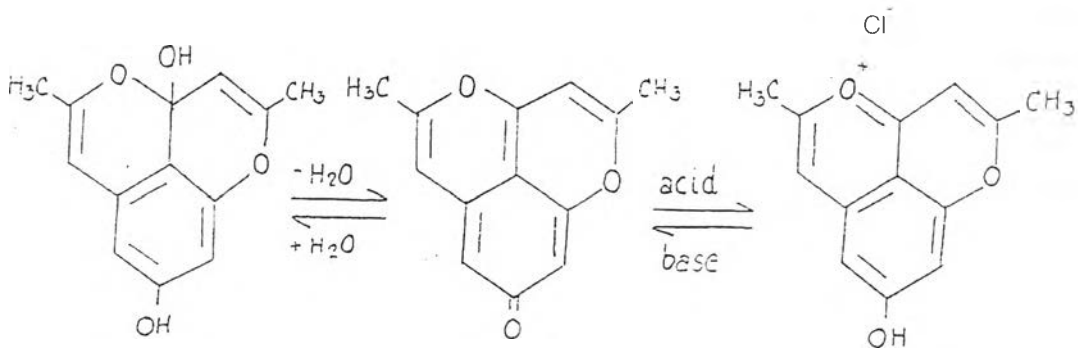
The reports from above suggested that barakol clearly has a sedative effect and decreases locomotor activity but the anxiolytic effect can not be explained.

Mechanism of the action of barakol

The conclusion of the mechanism of the action of barakol can be elaborated.

1. Effect on dopaminergic system

Barakol has been shown to increase rotation induced by apomorphine in rats with unilateral lesion in the substantia nigra with 6-hydroxydopamine (Jantarayota, 1987).



Barakol Anhydrobarakol Anhydronium salt

(Anhydrobarakol hydrochloride)

Figure 3 Conversion reaction among barakol, anhydrobarakol and anhydronium salt (anhydrobarakol hydrochloride).

The studies of the in vitro endogenous release of dopamine after incubation with barakol demonstrated that it is a potent inhibitor of K^+-stimulated endogenous dopamine release from striatal slices. The barakol induced inhibition of endogenous dopamine release occurred in a similar manner to dopamine D_2-like receptor agonist (pergolide and quinelorane) and this effect was prevented by dopamine D_2-like receptor antagonist (eticopride). The author concluded that barakol acts like D_2-like receptor agonist (Thongsaard, 1997a).

In the same study, the inhibition of barakol on dopamine release was not observed when the release of radiolabelled [^{3}H]-dopamine is measured under identical conditions to those used for assesment of endogenous dopamine release. A major different between the two experimental approaches could be that endogenous release essentially reflects release from newly synthesised store of dopamine which cannot be monitored by [^{3}H]-dopamine release. Following this suggestion it might imply that barakol has a selective effect on the newly synthesised dopamine (Thongsaard, 1997a).

2. Serotoninergic system

Barakol can suppress serotonin (5-hydroxytryptophan, 5-HT) induced head shakes in rats (Jantarayota, 1987).

Barakol can increased release of serotonin in hippocampus slice of rats (Thongsaard, 1997).

3. The study of binding sites of barakol in rat brains

The receptor autoradiography technique was used to detect and localize barakol ([^{125}I]-barakol) in any area of the rat brains. High grain density was observed in the caudate-putamen, accumbens nucleus, cerebral cortex, hippocampus, thalamic nuclei, granular cell layer of cerebellum, inferior colliculus and substantia nigra (Bhengsri, 1996).

The earlier reports, described above could not explain the mechanism of the action of barakol. It could not explain how barakol acts on any neurotransmitter in the brain but it might be that barakol acts on dopamine or serotonin in some area of the brain. In this study, the punching technique and HPLC-ECD were used to measure the concentrations of dopamine and serotonin in selective brain area in rats.

Micropunch technique

Micropunch (Punching) technique is microdissection of nuclei brain sections with hollow needle. Since its introduction in 1973 (Palkovitz, 1973) the technique has been adopted by serveral laboratories. It offers an order of magnitude with better structural resolution than was available previously, so neurobiologists have begun to focus on brain nuclei instead of heterogenous brain regions. The technique itself is rather simple and this is the most important reason for its popularity (Palkovitz, 1983). Microdissected brain nuclei can be used with other technique such as radio-immunoassay (RIA) (Fuhrmann et al, 1994), receptor binding technique (Meng et al., 1996), HPLC-ECD technique (Renner et al., 1986) etc. for many purposes.

HPLC-ECD technique

By 1975 liquid chromatographic instrumentation was described in the U.S. Pharmacopeia. Since that time, HPLC has become the most popular chromatographic technique in any research laboratory (Bidlingmeyer, 1993). The HPLC with electrochemical detection (ECD) has provided the neurochemist with a major tool to investigate the role of catecholamines and indoleamines in the peripheral and central nervous systems. These compounds, principally epinephrine (E), norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (serotonin, 5-HT) and their metabolites can be detected by their oxidation at a carbon-based electrode following separation on appropriate chromatographic column. This method provides a rapid, relatively cheap

and very sensitive assay for amines in small brain and plasma samples (Marsden, 1986).

The punching technique with HPLC-ECD was used to analyzed a concentration of catecholamines in any work such as those of Balthazart et al. (1989), Baumann et al. (1993), Lowry et al. (1996), Renner and Luine (1986) etc.

In this study, the microdissection (Punching) technique (Palkovitz, 1973) will be used to select the specific area in the brain and High-performance liquid chromatography with electrochemical detector (HPLC-ECD) will be used to measure the concentration of dopamine and serotonin in the brain area. So, if it can be proved that barakol acts by changing of dopamine or serotonin contents in central nervous system, the basic mechanism of barakol should be known. This result can be used for further studying on the mechanism of barakol and can be used to develop barakol as a drug to use in clinical levels in the future.