CHAPTER I

INTRODUCTION

Plants have broader uses than as just food and as a genetic reservoir. Increasingly they are the source of compounds useful for medicinal purposes. They have been found to synthesize various types of compounds to use as growth materials, protection against predators, infection, past, and disease for million of years (Cowan, 1999). This makes plants an excellent reservoir of medicines and chemical templates from which researchers can create new drugs and metabolites. Many chemical constituents of plants have been investigated and evaluated for their potential as pharmacologically active agents (Butler, 2004). Thus the number of researchs, developments and uses of natural products as therapeutic agents, especially those derived from plants, have been increasing in recent years (Rates, 2001).

In the course of our previous research, the chemicals of the stem bark of *Croton oblongifolius* Roxb. from Kui buri, Prachuap Khiri Khan province, Thailand, have been isolated. We afforded many kaurane diterpenes, among them; large amounts of *ent*-kaurenoic acid , *ent*-kaur-16-ene-19 oic acid, was isolated and its biological properties on cytotoxicity and inhibition of Na^+, K^+-ATPase activity were reported (Sirimongkhon, 2000). *Ent*-kaurenoic acid was also reported as a compound which could be isolated in a good yield from many plants such as those belonging to *Xylopia*, *Wedelia*, *Copaifera* and *Annona* spp. (Cavalcanti et al., 2006). Many reports revealed that this compound exerts a number of biological activities such as anti-parasitic, anti-tumor (Hanson, 1995) and anti-microbial activities (Velikova et al., 2000). Furthermore, the other kaurane type diterpenoids have also been reported as compounds which possess many kinds of interesting biological activities such as anti-microbial, anti-parasitic, insect anti-feedant, and anti-inflammatory activities (Rezende et al., 2000). Recently, significant inhibition of HIV replication in H9 lymphocytic cells has also been observed (Wu et al., 1996). Because of the valuable biological activities of the kaurane constitute it was considered to be worthy of further study. This is indicated by the continuously release of publications reporting on kaurane type diterpenoids.

Due to the ready availability from natural sources of the *ent*-kaurenoic acid, its isolation and its biological activities, it is interesting to use this compound as a starting material for the synthesis of derivatives in order to produce novel and /or compounds of higher biological activity. The hydroxylated kaurane derivatives are of special interest because they usually exhibit higher levels of biological activities than their parent compound (Vieria, Takahashi and Boaventura, 2000). However, the introduction of the hydroxyl group into the skeleton of kaurenoic acid is very difficult to achieve using classical chemical means due to their rigid structures. Additionally, some chemical reactions exhibit low specificity and/or require extreme reaction conditions for catalysis. Thus microbial transformations have been explored to over come these obstructions. In successful difficulties steroid biotransformation (Fogarty and Kelly, 1990; Mahato and Marjumda, 1993; Miller, 1985), microorganisms have been used as biocatalysts in order to incorporate the hydroxyl group into the skeleton of steroid compounds with good specificity and under mild reaction condition (Prave et al., 1987). Due to the similarly of the structures of steroid and kaurane diterpenoid, this method could be an alternative way for producing the hydroxylated kaurane diterpenoid including *ent*-kaurenoic acid.

Many reports have extensively shown that biotransformation is a powerful methodology for introduction of the hydroxyl group into the skeleton of *ent*-kaurenoic acid (Hanson, 1992). It has been achieved with various types of microorganisms. The literature reviews suggested that fungi are the most effective microorganisms available and are of considerable use in biotransformations for the partial synthesis of the hydroxylated compounds (Beilby et al., 1973; Ghisalberti et al., 1977; Silva et al., 1999). Not only if the desired product could be produced but also if the various kinds of reaction performed by fungi could be elucidated then the researcher could gain more data for selecting those fungi which perform the desired reactions. Accordingly, finding fungi with the specific hydroxylation activities becomes one of the important steps to improve future work in this field. Many fungi have been reported in the biotransformation process for introducing the hydroxyl group into the kaurenoic acid skeleton but the Psilocybe mushroom has not been used before in this process.

Psilocybe cubensis, a mushroom belong to the genus *Psilocybe*, has been reported as the mushroom which presents high capacity to introduce the hydroxyl group into the intermediate to form the hallucinogenic indol alkaloids, psilocin and

psilocybin (Gartz, 1989). It is interesting that *P. cubensis* probably could transform other compounds to form the hydroxylated products including *ent*-kaurenoic acid.

The information referred to above encouraged us to attempt the modification of the structure of *ent*-kaurenoic acid to produce the hydroxylated kaurane derivative through the agency of *P. cubensis*. Therefore, this study will describe the bio-transformation of *ent*-kaur-16-en-19-oic acid by *P. cubensis* and report on the enzyme involved in the biotransformation process.

The objectives of this research are as follows:

1. To investigate the biotransformation of *ent*-kaurenoic acid by *Psilocybe cubensis*.
2. To identify the transformation products and determine their biological activities.
3. To study the enzyme in *Psilocybe cubensis* involved in biotransformation process.