CHAPTER I

INTRODUCTION

The term 'natural product' defines a vast array of naturally occurring organic compounds which have been organized into groups of substances, like the alkaloids, terpenes, aromatics, lipids, peptides and others, depending on chemical and structural features, and on their biosynthetic origin. Many natural products are biologically active, and most of these have complex structures possessing multiple functional groups. These features render routine chemical and biochemical manipulations difficult, alternative is to produce them though chemical derivatization of natural compounds. This may require functionalization at non-activated carbon atoms. This type of transformation is very difficult to be achieved with reasonable yields and regioselectivity using classic chemical reactions. Also biocatalysis is suggested as an approach for solving problems of special interest to natural products chemistry [1].

Bacteria, fungi and other microorganisms are well equipped to serve as biocatalysts in natural products chemistry. Such organisms thrive through their abilities to alter metabolically the structures of a diverse range of organic compounds. Microbial enzymes may perform reactions of biosynthetic or biodegradative importance to the cell; they may also cause biotransformations which confer little apparent benefit to the microorganism containing them. All of these types of enzymes may serve as useful catalysts in transforming compounds natural to the cell or with xenobiotics [2].

As a continuation of our investigation of bioactive compounds from Plao-yai (*Croton oblongifolius*), which had been used in Thai folk medicine as tonic, inhibit chronic enlargements of livers, dysmenorrhea, purgative and dysentery [3], specimens from various locations in Thailand have been investigated for their chemical constituents and biological activities. It was found that the main components of each specimen were different [4-6]. *ent*-Kaur-16-en-19-oic acid, isolated from the stem bark of *C. oblongifolius* collected from Amphoe KuiBuri, Prachuap Khiri Khan Province, possessing several biological activities including plant growth regulators [7], and Na^+,K^+-ATPase inhibitor which exhibits an IC_{50} of 2.2×10^{-5} M against crude enzyme Na^+,K^+-ATPase from rat brain [8]. Moreover, *ent*-1,2-dehydro-3-oxomanoyl oxide was discovered from specimen collected in Loei Province. They have been

shown to possess cytotoxic activities against P388 cells line and five tumor cell lines including Hep-G2 (hepatoma), Chago (lung), SW620 (colon), Kato-3 (gastric) and BT474 (breast) [9].

The use of microorganisms to achieve the biotransformation of pharmacologically active compounds provides a convenient and economic means of obtaining compounds which would be very difficult or expensive to produce chemically. Furthermore, the advantage of microbial transformation instead of conventional synthetic methods relies upon the high specificity of enzymatic reactions. This technique makes it possible to hydroxylate substrate regioselectively in single step. In recent years, a considerable number of studies exploring the microbial hydroxylation of some diterpenoids have been reported furnishing novel compounds in good yields. The hydroxylation pattern of these bioactive compounds may influence their binding on to the receptors, as was proposed for the diterpenoids [10]. Therefore, these biotransformation processes have been used to introduce hydroxyl groups, at positions difficult to achieve by chemical means on the substrates or it may be used to may prepare novel highly oxygenated diterpenoids useful in biological screenings. From the results of biotransformation experiments on kaurane, labdane and their derivatives carried out by previous reports [11-20] and our research group [16] encouraged us to prepare novel derivatives for biological assays. In this research, we describe the biotransformation of *ent*-kaur-16-en-19-oic acid and *ent*-1,2-dehydro-3-oxomanoyl oxide by whole cells of various fungal microorganisms that extensively studied in steroid and terpenoid hydroxylation.

Thus, the objective of this research is summarized as follows:

1. To investigate the biotransformation of diterpenoids by some fungi.
2. To identify the microbial transformation products.
3. To determine the biological activity of the microbial transformation products.