CHAPTER III

OBJECTIVES

The aim in this research work is to study the biochemical properties of GG18H and its CPR partner enzyme present in the plant of for *C. stellatopilosus* Ohba. This plant accumulates the acyclic diterpenoid plaunotol as its major constituent, and GG18H is presumably the enzyme catalyzing the last step of pluanotol biosynthesis. Since it is well known that the enzymes involved in hydroxylation reaction are membrane-bound P450 enzyme, their characterization is complicated.

Therefore, gene cloning and expression of GG18H and its pair of CPR are necessary as initial step of this study. This is followed by the study on the relationship of the target genes with various plant P450s in terms of protein sequence and phylogenetic analysis. In addition, the transcription level of the genes related to the level and accumulation of plaunotol is also of interest which can be studied by real time PCR techniques.

Specifically, the objectives of this study are as follows:

1. To clone genes of an acyclic diterterpene hydroxylase and its related cytochrome P450 reductase from *C. stellatopilosus* Ohba
2. To characterize the genes encoding the two enzymes in terms of classification and phylogenetic relationship
3. To examine the expression and regulation of the acyclic diterterpene hydroxylase and cytochrome P450 reductase in *C. stellatopilosus* Ohba by real-time PCR technique
4. To characterize the enzyme properties and identify the natural substrates of the recombinant enzymes