CHAPTER I

INTRODUCTION

In the photosynthetic pathway, plants capture solar energy to converse carbon dioxide and water into carbohydrate and oxygen. The carbohydrate provides the basic material for the biosynthesis and through controlled oxidation, the driving energy for mantain complicate metabolism and perpetuate life. These primary metabolic processes are vital importance for living state and are essential for all organisms. Unlike primary metabolism, the secondary metabolic processes are the synthesis and catabolism of endogenous compounds by specialized enzymes (Luckner, 1971). The results of these processes lead to diverse groups of chemical complexity (Luckner, 1990). These secondary metabolites are an expression of the cell specialization, which is either triggered by the process of cell expression or represent an aspect of the process of plant development (Endress, 1994). In contrast to the primary metabolites, individual secondary compounds vary widely in their distribution between plant species and some may occur only in a single or a few related species and they also even large difference in enviromental conditions (Rhodes, 1994). With respect to the natural functions of secondary metabolites, it has been shown that these compounds are involved in the defense mechanism against herbivores and microorganisms (Enyedi, 1992; Wink, 1988; Decosmo, 1985), or some as selective attractant and others are served as photoprotectants or as toxins.

Secondary metabolites have been used by man in various ways since anceint times, mostly by trial and error, experience, gain the knowledge and passed from generation to generation. Nowadays, secondary metabolites are used as dyes, poisons, fragances and drugs. The current research in this area is focusing on isolation of plant secondary metabolites with pharmacological activities (Hamburger and Hostettman, 1991). These have led to a rapid development of knowledge on different aspects of secondary metabolism and prompted man to cultivate the producing plants.

In 1960, a picture of many biosynthetic pathways of various secondary metabolites began to emerge, largely by speculation based on isotropically labelled precursor feeding experiments to various plants (Zenk, 1991). Although these tracer techniques can provide ideas about the possible plant biosynthetic routes, they do not reveal the actual sequence and mechanism of the biosynthesis of the compounds. The powerful support for any biosynthetic pathway as detail information on the reaction involved may be gained by isolation, purification and characterization of the enzymes which catalyse individual steps of the biosynthesis (Herbert, 1981). Once the biosynthetic pathways involved are known and their enzymes are isolated and characterized, one can clone the genes involved in the pathways. Subsequently, the regulation of the secondary metabolism can be studied at the level of gene. Eventually, genetic engineering approches can be considered to improve the production of secondary metabolites (Verproote, 1993).

Among the various groups of secondary metabolites, alkaloids are the pharmacologically important ones. Isoquinoline alkaloids have been used in pharmacotherapy since prehistoric period. They are present in many plants. It has been shown that 2,500 known structures of isoquinoline alkaloids are derived from (*S*)-norcoccluarine (Kutchan, 1998). Many studies have provided information on the specific enzymes which are involved in various steps of the biosynthetic pathways of protoberberine (Schinder and Zenk, 1992), morphenan (Kutchan *et al.*, 1991), benzophenanthridine (De-Eknamkul *et al.*, 1992), protoberberine (Philipson *et al.*, 1985) and so on. Unlike most isoquinoline structures, emetine alkaloids which have been detected in the families Rubiaceae (*Cephaelis spp.*) and Alangiaceae (*Alangium spp.*) are believed to originated from alkaloidal glucosides which are similar to the indole alkaloid groups. The study of the biosynthetic pathways of emetine alkaloids was also originally based on precursor feeding experiments (Nakagura, *et al.*, 1978). The experiment has been provided information on the first step of emetine biosynthesis which involves the condensation of dopamine and secologanin (monoterpene glucoside) in both *Cephaelis spp.* and *Alangium spp.* (Figure 1). It has been shown that the two precursors are condensed in a Pictet-Splenger manner to form two

epimers, (*R*)-deacetylipecoside which is subsequently converted into alangiside-type glucosides and (*S*)-deacetylisoipecoside which lead to protoemetine intermediate and subsequently tranformed to isoquinoline monoterpene alkaloids such as emetine, cephaeline, tubulosine. Although the biosynthetic pathways of these tetrahydroisoquinoline monoterpene alkaloids have been proposed, very little is known about the involved enzymes with stereochemical control. Recently, the first step of this biosynthetic pathway has been reported. In this study, two novel enzymes activities have been found to be involved in the condensation of dopamine and secologanin in the cell-free extracts prepared from the leaves of *Alangium salviifolium* Wang (De-Eknamkul, *et al.*, 1997). The condensation produced two epimers, (1*R*)-deacetylipecoside and (1*S*)-deacetylisoipecoside. The discovery of these two enzyme activities has suggested that the naturally occurring (*S*)- and (*R*)-forms of various tetrahydroisoquinoline monoterpene alkaloids are determined by the first step of the biosynthesis (De-Eknamkul, *et al.*, 1997).

In an attempt to study the biosynthesis of tetrahydroisoquinoline monoterpene alkaloids at molecular level and to understand about the organization of genes involved in the biosynthetic pathway, the enzymes of specific pathway must be identified and characterized. It was nesscessary to isolate and purify enzymes to clearify the complex regulation of its biosynthetic pathway (Kutchan *et al.*, 1991).

This study is a continuation of the previous work carried out in our laboratory (De-Eknamkul, *et al.*, 1997). It involves purification and characterization of the first enzyme of the pathway for dopamine and secologanin condensation. This step of investigation is extremely important since it leads to molecular biological studies of this alkaloid group which have not yet been done before. The results obtained from this work are expected to be as important as the well-known enzyme strictosidine synthase (SSS, EC 4.3.3.2) of the indole alkaloids pathways.