CHARPTER I

INTRODUCTION

The production of secondary metabolites in higher plants is a complex process which involves not only the complete expression of their biosynthetic pathways but also the availability of cellular spaces for accumulation of the products (Verpoorte, Heijden and Schripsema, 1993). During the last four decades, biosynthetic pathways of various secondary products have been proposed based on the information obtained from feeding experiments. However, such tracer techniques can only provide ideas about the possible biosynthetic routes, They do not reveal the actual sequence and mechanism of the biosynthetic process in detail (Phillipson, Roberts and Zenk, 1985). Presently, it is widely accepted that the ultimate proof that a biosynthetic pathway is correct can only be obtained from studies with purified enzymes which catalyze various steps of secondary metabolite biosynthesis.

Recently, a number of biosynthetic enzymes involved in the biosynthetic pathways of some isoquinoline alkaloids have been studied. These alkaloids include protoberberines (Schneider, and Zenk,1992), morphinans (Kutchan *et al.*, 1991), benzophenanthridines (De-Eknamkul *et al.*, 1992), and protopines (Phillipson *et al.*, 1985) and so on. Among the isoquinoline alkaloids, the emetine group is considered one of pharmaceutically-important drugs. However, very little is known about the enzymes of their biosynthetic pathways. Based on various feeding experiments carried out previously (Nagakura, Hofle, and Zenk, 1978), it has been proposed that the first step of emetine biosynthesis involves the condensation of dopamine and secologanin (Figure1). According to this hypothesis it is possible that the condensation products can be both deacetylisoipecoside (S-configuration) and deacetylipecoside (R-configuration). This possibility was proposed based on the fact that some natural alkaloids isolated from emetine-containing plant such as *Alangium salviifolium* Wang.

alkaloids isolated from emetine-containing plant such as *Alangium salviifolium* Wang. possess both S-configuration at C-1 (eg emetine and cephaeline) and R-configuration (eg ipecoside and alangiside) (Cordell *et al.*, 1989). The different C-1 configuration of these alkaloids has been thought to occur at the first condensation step (Figure 1) (Nagakura *et al.*, 1978). However, the enzymes catalyzing these condensation reactions have not yet been found.

In order to clarify the reaction of the first step of dopamine and secologanin condensation, it is necessary to find the enzyme(s) responsible for the catalysis. Once the enzyme is found, it is possible to carry out experiments *in vitro* , in the presence of it substrates, to detect the formation of the reaction product. Identification and characterization of the product and its configuration would lead to a better understanding of the first enzymatic step of the pathway. Therefore, the objectives of this study are to search for the dopamine-secologanin condensing enzyme and to characterize the product of the enzymatic reaction.

In Thailand, the plant of *A. salviifolium* can be found easily. This plant has been used in folkloric medicine for treatment cough and diarrhoea. It has been reported to have high content of tetrahydroisoquinoline monoterpene glucosides (Cordell *et al.*, 1989). *A. salviifolium* was, therefore, chosen as plant material for this study. Since plant tissue and cell cultures have been used successfully for evaluating the biosynthetic enzyme pathways of alkaloids (Kutchan and Zenk, 1993), we also established callus and suspension cultures of this plant.

In this thesis, the potential of *A. salviifolium* callus and suspension cultures in the production of the emetine alkaloids were first studied. The results obtained were used for comparison among various plant materials for choosing suitable enzyme source. Finally, enzyme extracts were prepared from the selected material and used for enzyme detection followed by identification and characterization of the enzymatic product.

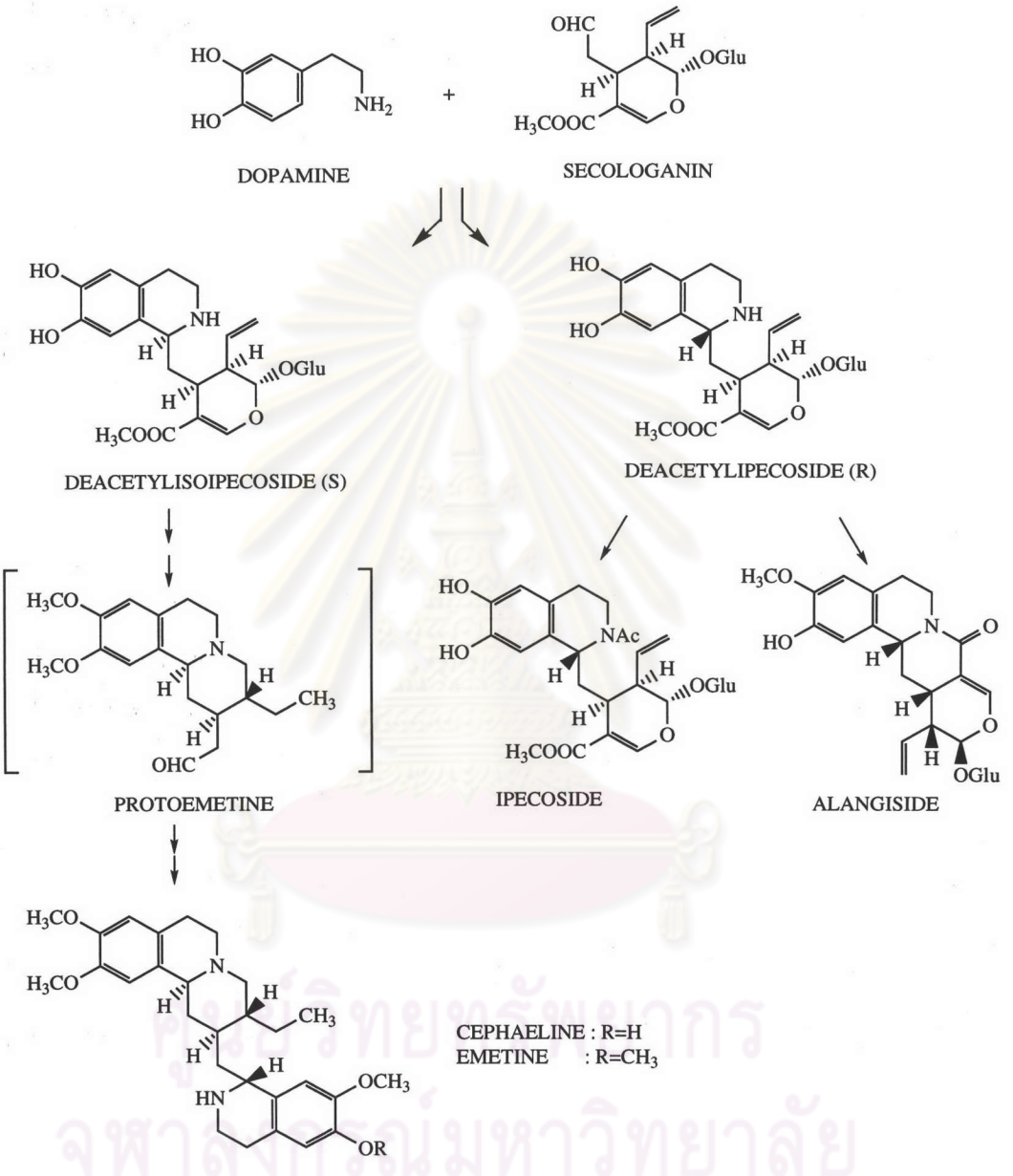

Figure 1 Proposed biosynthetic sequence for the biosynthesis of cephaeline, emetine and the alkaloidal glucoside and alangiside.