CHAPTER I

INTRODUCTION

There are numerous groups of alkaloids isolated from the plant kingdom. Among these, the isoquinoline alkaloids have played an important part in the development of the chemical and biological sciences. Among the isoquinoline alkaliods, aporphines are considered the largest group (Cordell, 1981).

Like the other isoquinolines, the group of aporphines has been proposed to be derived from either *(S)*-or *(R)*- reticuline, the alkaloid with tetrahydrobenzyl-isoquinoline nucleus (Bhakuni, 1977; Luckner, 1990; Shamma and Maniot, 1978). However, up till now, the biosynthesis sequences from reticuline are still uncompletely known. A number of possible routes for the biosynthesis of aporphines from reticuline have been proposed based on previous feeding experiments using radioactively labelled precursors (Cordell, 1981). However, biosynthesis studies at the enzymatic level have hardly been touched.

Although, *in vivo* feeding techniques are neccessary to provide first ideas about a biosynthetic pathway, they do not reveal the actual sequence and mechanism of the biosynthetic process in detail. In fact, the ultimate elucidation of a biosynthetic sequence needs information on the structures of enzymatically formed intermediats and the characteristics of various enzymes involed in the pathway. This information can only be obtained by enzymological studies of the biosynthetic enzymes. However, the expression level of secondary pathways in higher plants appears to be very low in general. Also, the presence of hight content of phenolic compounds and other undesirable cell constituents tends to inactivate these enzymes during the isolation procedure (Loomis and Battaile, 1966). As a result, the use of intact plants for enzyme isolation is generally unsuccessful.

Plant cell cultures, capable of producing desired secondary products, are an excellent source of enzyme systems. Culture cells and callus can be grown under exactly defined conditions on a large scale, thus providing any desired amount of homogeneous plant material as enzyme source independent of seasons. Furthermore,

Figure 1 *Stephania pierrei* Diels

A. leaf B. tuber

C. inflorescence

D. climber stem

levels of catalytically active proteins are higher in culture cells as compared with whole plants because the time required for product synthesis under cell culture conditions is much shorter than in the field-grown plants (Zenk, 1985). Therefore, a number of reports have been published currently on the enzymological studies of biosynthetic pathways of secondary metabolites using plant cell and tissue cultures.

With the lack of information on the aporphine alkaloid biosynthesis in higher plants, we searched for a plant species which contain high aporphine alkaloids as a model for the study. Among various aporphine-containing plants, *Stephania pierrei* Diels is of most interest since it accumulates high contents of eleven aporphines, seven protoberberines, one morphinan alkaloid, one hasubanan alkaloid and three tetrahydrobenzylisoquinolines including reticuline (Likhitwitayawuid, *et al*, 1993). *S. pierrei* or "kling-klaang-dong" (กลิ้ง–กลาง–ดง) or "bhoraphet - phung - chang" (บอระเพ็ดพุงช้าง) in Thai, belongs to the family Menispermaceae (เต็ม สมิตินันท์, 1980). Its tubers have been used as stimulating and aphrodisiac in Thai folkloric medicine (เสงี่ยม พงศ์บุญรอด, 1979). The ethanolic extracts of *S. pierrei* have been tested and found to process cytotoxic and antimalarial potential (Likhitwitayawuid, *et al.*, 1993b).

The presence of both reticuline and aporphine alkaloids in *S. pierrei* makes this plant an ideal material for biosynthetic study of reticuline-aporphine alkaloid pathway. Therefore, in this study, tissue cultures of *S. pierrei* were established and studied their potential in the production of aporphine alkaloids. The results obtained from the study will be used for evaluating *S. pierrei* cultures for further investigation at the enzymatic level which, hopefully, will lead to a better understanding of the biosynthetic pathways of aporphine alkaloids in higher plants.