CHAPTER I

INTRODUCTION

There is a continued commercial demand for a wide range of secondary compounds, particularly in the food and pharmaceutical industries. The fact that plant cell cultures are not utilized to any great extent industrially for the direct production of such compounds is due to economic reasons. One of the problems associated with plant cell cultures is the usually low obtained yields in the production of some particular secondary products.

Generally, the production of secondary metabolites in plants is a complex process highly coordinated in space and time. Its main components are biosynthesis and accumulation which are usually modified by tissue- and cell-specific compartmentation. Depending on the developmental stage, transport and degradation can be additional factor. In view of the complexity of secondary metabolism, it is not surprising that most cell culture systems have failed to produce a given compound in large quantities. Thus, it is exciting that several cell cultures have been established which produce a higher amount of a secondary metabolite than the respective intact plants (Ellis, 1988). Most successful compounds are synthesized by root tissue and the site of accumulation is either within the producing cell or the neighboring cells. On the other hand, compounds synthesized in roots, which are transported *via* the xylem to other plant parts, usually fail to accumulate in plant cell suspension cultures. This means that secondary metabolites whose biology shows a higher degree of complexity are usually not yet successfully produced by plant cell cultures (Wink, 1987). It is interesting that when cultured plant cells differentiate into tissues and organs, their capacity to produce the secondary metabolites set in immediately. Thus, a possible approach to increase yields of secondary compounds is to allow differentiation of tissue by production of shoot or root cultures.

A detailed understanding of the enzymatic formation of secondary products, however, is a prerequisite for rational strategies to improve product formation. It is essential

that their control and regulation mechanisms can be understood if they are to be manipulated. To manipulate plant secondary metabolism according to our needs, we have to understand the basic biochemical principles of product formation. Plant cell cultures have proved to be useful sources of enzyme which catalyze specific biosynthetic steps. Thus, this could be accomplished by using enzymology techniques which involve purification and characterization of the biosynthetic enzymes.

Among various secondary metabolic pathways in plants, the biosynthesis of naphthoquinone is of particular interest. There are at least four different routes of naphthoquinone formation established in the higher plants, including *p*-hydroxybenzoate, homogentisate, *o*-succinylbenzoate and polyketide pathways. In this study, emphasis was put on the naphthoquinones that accumulated in *Impatiens balsamina* L. Lawsone (2-hydroxy-1,4-naphthoquinone) and its methyl ether, 2-methoxy-1,4-naphthoquinone are the two main naphthoquinones found naturally in *I. balsamina* (Little *et al.*, 1948; Bohm and Towers, 1962). Both compounds have been reported to exhibit strong antifungal activity (Tripathi, Srivastava and Dixit, 1978; Farnsworth and Cordell, 1976; Thatree Phadungcharoen *et al.*, 1988). Biosynthetically, it has been proposed based on feeding experiments that lawsone is formed in plant *via* *o*-succinylbenzoic acid (Dansette and Azerad, 1970; Grotzinger and Campbell, 1974), a key intermediate arising from chorismic acid and α-ketoglutarate (Chen and Bohm, 1966; Grotzinger and Campbell, 1972). However, none of the enzyme involved in the formation of the naphthoquinones has been found in plants. This prompted us to investigate the biosynthetic pathway of lawsone and its methyl ether. We first examined for a suitable enzyme source by establishing various type of *in vitro* cultures of *I. balsamina* (Pharkphoom Panichayupakaranant and Wanchai De-Eknamkul, 1992). It was found that the root cultures of *I. balsamina* could produce a number of natural products, mostly naphthoquinone and coumarin derivatives. In addition, the content of lawsone in the root culture was found to be higher than in the intact plants. This type of culture is therefore a suitable source of enzyme for the study on the biosynthesis of lawsone and its methyl ether.

As part of our interest in investigation of the biosynthetic pathway of lawsone and its methyl ether, we first aimed to know the type and content of secondary metabolites produced in the root cultures. Thus, the isolation and structural elucidation of the secondary compounds produced by the root cultures were firstly performed. Consequently, the potential of the root cultures as a suitable enzyme source was investigated by the study on the chemical patterns and contents of the isolated compounds compared with those of the intact plant. For this aspect, the kinetic of growth and the formations of naphthoquinone and coumarin derivatives were also examined. Finally, the potential enzyme system involved in the biosynthesis of lawsone and its methyl ether was examined by both *in vivo* feeding and *in vitro* cell-free system with isotropic precursors, followed by partial purification and characterization of the enzyme.