CHAPTER I

INTRODUCTION

Polyketides are a large family of natural products found in bacteria, fungi and plants, and include many clinically important drugs. They are biosynthesized from acyl-CoA precursors by polyketide synthases (PKSs) (Shen, 2003). In plants, polyketides are biosynthesized by type III polyketide synthases. Type III PKSs catalyze the sequential decarboxylative condensation reaction of malonyl-CoA and intermediates from acetate and shikimate pathways. This chain extension is followed by cyclization of the linear polyketide intermediates, producing a variety of compounds including chalcones, dihydrochalcones, stilbenes, acridones, pyrones, xanthones, benzalacetones (Schröder, 2000; Austin and Noel, 2003) and chromones (Abe et al., 2005b).

Plant type III polyketide synthases playing roles in the biosynthesis of compounds are chalcone synthase (CHS), pyrone synthase (2-PS), stilbene synthase (STS), bibenzyl synthase (BBS), homoeriodictyol/eriodictyol synthase (HEDS), acridone synthase (ACS), benzylphenone synthase (BPS), phlorisovalerophenone synthase (VPS), coumaroyl triacetic acid synthase (CTAS), benzalacetone synthase (BAS) (Schröder, 2000; Austin and Neol, 2003), aloesone synthase (ALS) (Abe et al., 2004), pentaketide chromone synthase (PCS) (Abe et al., 2005b) and octaketide synthase (OCS) (Abe et al., 2005a).

Chalcone synthase (CHS) and stilbene synthase (STS) catalyze three condensation reactions of a phenylpropanoid CoA-ester with three acetate units from malonyl-CoA to synthesize a tetraketide intermediate and produce naringenin

chalcone and resveratol (Schröder, 1997; 2000; Austin and Neol, 2003). Acridone synthase (ACS) uses *N*-methylanthranioyl-CoA and three malonyl-CoA to produce acridone (Lukačin et al., 1999). Phlorisovalerophenone synthase (VPS) uses linear Co-A ester (isovaleryl-CoA) as starter substrate and three malonyl-CoA (Zuurbier et al., 1998; Paniego et al., 1999). Pyrone synthase (2-PS) uses acetyl-CoA and two condensation reactions with malonyl-CoA to form the pyrone backbone (Eckermann et al., 1998). Benzalacetone synthase (BAS) catalyzes a one-step decarboxylation of *p*-coumaroyl-CoA with malonyl–CoA to produce benzalacetone (Borejsza-Wysocki and Hrazdina, 1996). Aloesone synthase (ALS) catalyzes six condensations of malonyl-CoA and subsequent cyclization to yield an aromatic heptaketide, aloesone (Abe et al., 2004). Pentaketide chromone synthase (PCS) catalyzes five condensations of malonyl-CoA to produce a chromone (Abe et al., 2005b). For the novel type III PKS, octaketide synthase (OCS) catalyzes eight condensations of malonyl-CoA to produce SEK4 and SEK 4b (Abe et al., 2005a). The diversity of functions with divergent products are based on different substrate specificities, variation in the number of the condensation reactions, mechanism of cyclization reaction and steric modulation of type III PKS structure (Schröder, 2000; Austin and Noel, 2003).

Plumbago indica Linn (Plumbaginaceae) is a perennial shrub distributed mainly in the tropical region. The roots of this plant are rich in plumbagin. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) is an important naphthoquinone showing a broad range of biological activities i.e. antimalarial (Likhitwitayawuid et al., 1998), antimicrobial (Didry et al., 1994; Ribeiro de Paiva et al., 2003), anticancer (Fujii et al., 1992; Kuo et al., 1997; Lin et al., 2003) and antifertility actions (Kubo, Uchida and Kloke, 1983). In higher plants, plumbagin has been found in *Plumbago* spp. (Van Der Vijver, 1972), *Drosera* spp. (Zenk et al., 1969), *Drosophyllum* spp. (Zenk et al., 1969), *Diospyros* spp. (Mallavadhani, Panda and Rao, 1998), *Nepenthes* spp.

(Cannon et al., 1980; Likhitwitayawuid et al., 1998; Aung et al., 2002) and *Ancistrocladus* spp. (Anh et al., 1997; Bringmann et al., 1999). Its biosynthesis has been proposed to involve the polyketide (acetate/malonate) pathway, based on results from various feeding experiments (Duran and Zenk, 1971; Bringmann et al., 1998). In plants of the genera *Drosera* and *Plumbago*, plumbagin has been demonstrated to arise from a hexaketide, rather than the shikimate pathway, which is used in the biosynthesis of juglone and menadione. Feeding experiments with [1-^{14}C, 2-^{14}C-acetate] and 2-^{14}C-malonate heavily labeled this naphthoquinone, suggesting that plumbagin is formed by the well-known polyketide pathway (Duran and Zenk, 1971). Based on these results, the possible biosynthetic pathway of plumbagin has been proposed as shown in Figure 1. Acetyl-CoA is first condensed with five acetyl residues derived from malonyl-CoA to form a hexaketide intermediate via condensation reaction. The folding of this intermediate molecule can lead to plumbagin. The polyketide synthase should be involved in plumbagin biosynthesis. At the present, the polyketide synthase has not yet been discovered in plumbagin-producing plants. We, therefore propose to study this enzyme and gene of polyketide synthase in *P. indica*. Information obtained from the study will lead to the understanding of the plumbagin biosynthesis and the evolution of various genes with polyketide-producing plants.

In this research work, plumbagin-containing tissue cultures of *P. indica* are established for obtaining materials for enzymological studies. Crude protein extracts are prepared from the *P. indica* tissue materials and detected for their polyketide synthase activity involved in the biosynthesis of plumbagin. Finally, molecular cloning techniques are used to clone and express a polyketide synthase gene with its product identification.

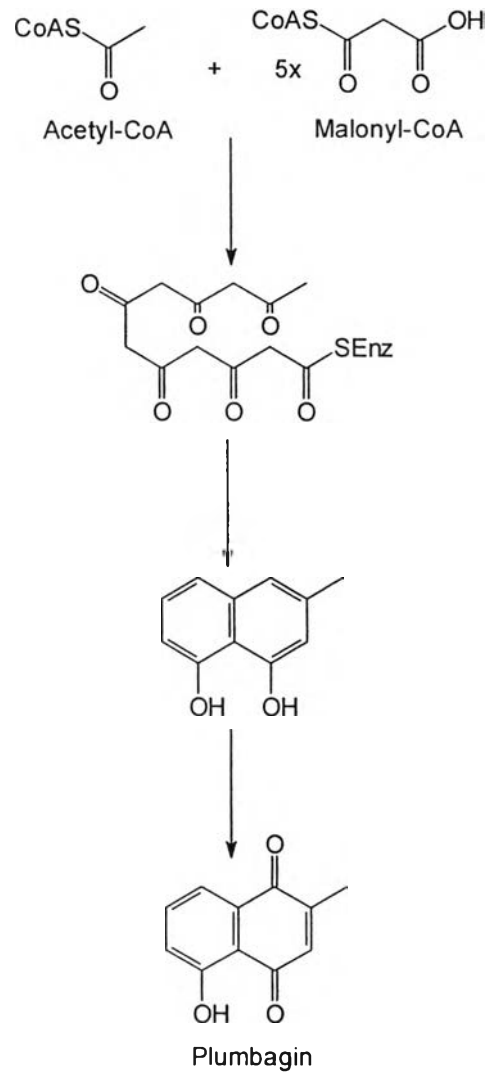

Figure 1 Proposed biosynthetic pathway of plumbagin