CHAPTER I

INTRODUCTION

Croton sublyratus Kurz. or Plaunoi (Thai-name) (Figure 1) is a plant belonging to the family Euphorbiaceae. It grows in the tropical areas, especially those nearby the Andaman Sea such as Indonesia, Malaysia, Thailand, Myanmar and Southern China (ณรงค์ เพ็งปรีชา, 2530). In Thailand, *C. sublyratus* is mostly found in the provinces of Prachin Buri, Prachuap Khiri Khan and the border between Myanmar and Thailand (ณรงค์ เพ็งปรีชา, 2530; ลีนา ผู้พัฒนพงศ์ และ ธวัชชัย วงศ์ประเสริฐ, 2530; วีณา วิรัจฉริยากุล และ คณะ, 2533;). In the past, the plant was used as a Thai folk medicine for anthelmintic and dermatologic agent for skin disease (Dhavadee Ponglux., *et al.*, 1987). Presently, *C. sublyratus* leaves have been well known as being raw material for manufacturing antipeptic-ulcer drug.

C. sublyratus has been reported to contain many diterpenes (Kitazawa *et al.*, 1979; Kitazawa *et al.*, 1980; Takahashi *et al.*,1980; Kitazawa *et al.* and Ogiso, 1981; Kitazawa *et al.*,1982). Among these, the most famous compound, which is the active antipeptic ulcer substance, is plaunotol (Ogiso *et al.*,1978).

Plaunotol (Figure 2) is an acyclic diterpene alcohol which was registered with the World Health Organization (WHO) under the code CS-684 (Ogiso *et al.*, 1985; Department of Medical Information, Sankyo Co, Ltd.,1993). It has been manufactured as a cytoprotective-type antiulcerative drug by Sankyo Co., Ltd. under the trade name of Kelnac®. Since attempts to synthesize plaunotol at a price competitive with the natural product have not so far been successful. The company has cultivated *C. sublyratus* in Thailand for leaf harvesting at Prachuap Khiri Khan

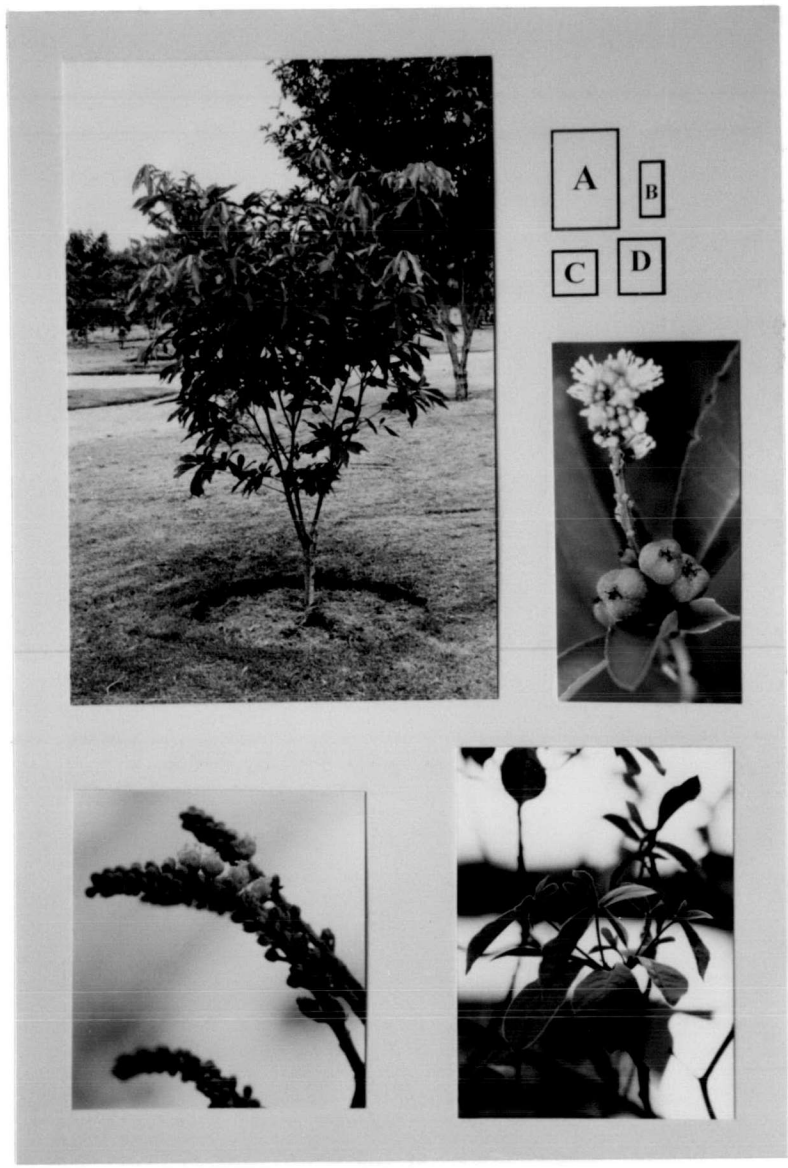

Figure 1 *Croton sublyratus* Kurz. (Euphorbiaceae) (A), Fruits (B), Inflorescences (C), Leaves (D)

with the area more than 7,000 rai. The plantation site has more than a million plants for supplying leaves for plaunotol extraction (ณรงค์ เพ็งปรีชา, 2530).

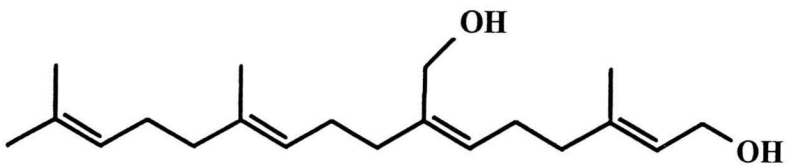

Figure 2 The chemical structure of plaunotol.

Although the structure of plaunotol has been known for almost twenty years, very little is known about its biosynthetic pathway in *C. sublyratus* plant. However, based on its structure, plaunotol seems to be a simple compound since it is a 18-hydroxy derivative of geranylgeraniol (GGOH), a common precursor of all natural diterpenoids. It is well accepted that GGOH is biosynthesized via the mevalonate/ terpenoid pathway and its immediate precursor is geranylgeranyl pyrophosphate (GGOPP). Therefore it is very reasonable to propose that plaunotol is biosynthesized from GGOPP by two steps of enzymatic reactions (Figure 3). First, the pyrophosphate was hydrolysed from GGOPP by the enzyme phosphatase to form GGOH . Second, GGOH is hydroxylated at C-18 position by the enzyme 18-hydroxylase to form plaunotol. Until now, there has been no report to support this proposed pathway. One of the reasons may be that a suitable starting material for the study such as *C. sublyratus* cell cultures producing high content of plaunotol has not yet been available. Another reason may be the difficulty in searching for the specific hydroxylase enzyme which is usually complex, membrane bound and unstable.

Due to the available of *C. sublyratus* plant in Thailand, we decided to search for the enzyme geranylgeraniol-18-hydroxylase in *C. sublyratus*. The leaf has been chosen as a material for the study since it is the potential biosynthesis site of plaunotol and it is abundant. The results from this study are expected to clarify the biosynthesis of plaunotol in *C. sublyratus* and to examine the possibility for biotechnological application of the enzyme in plaunotol synthesis.

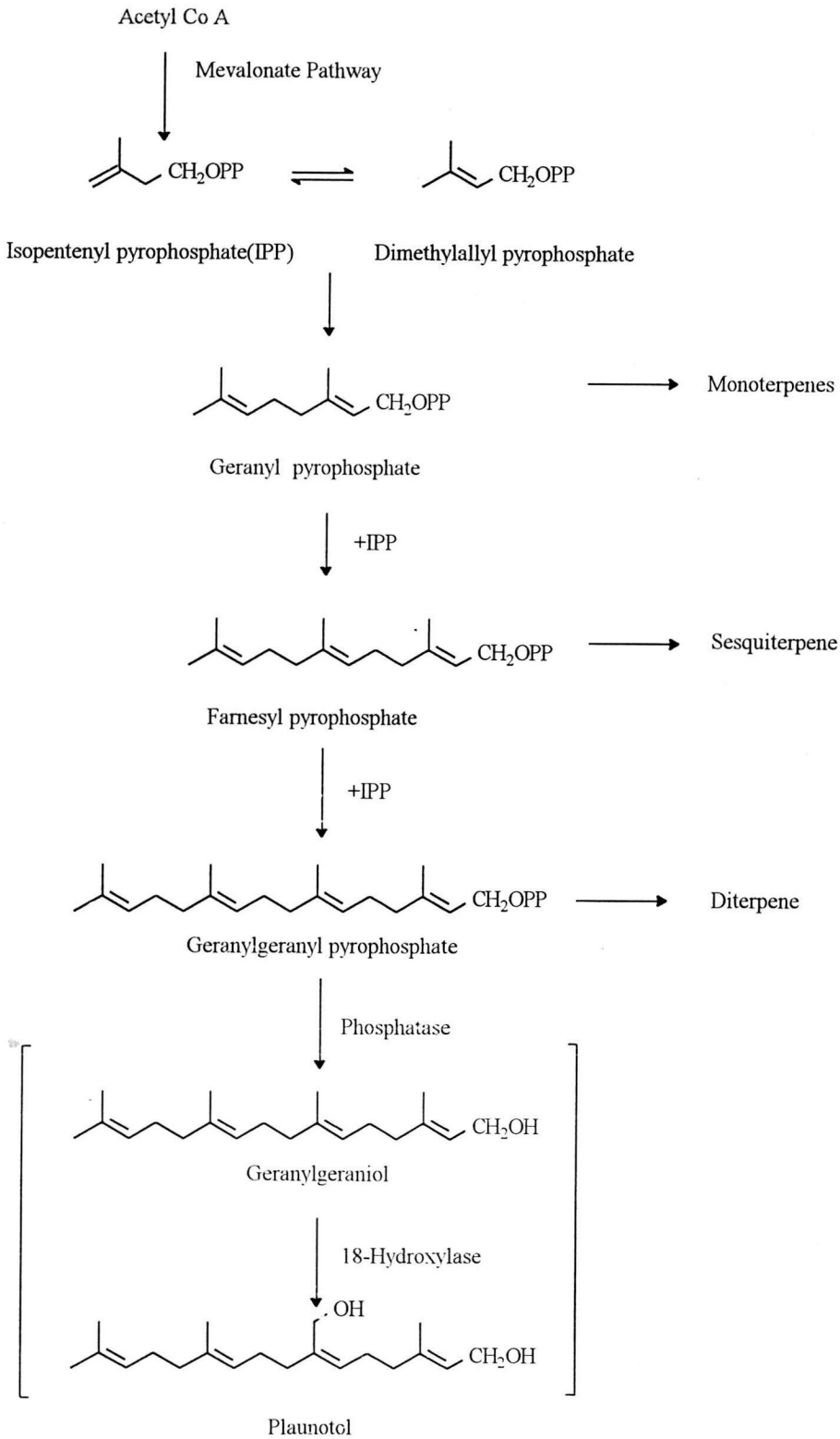

Figure 3 Proposed biosynthesis of plaunotol in *C. sublyratus* Kurz.