CHAPTER I

INTRODUCTION

Animals, plants, and micro-organisms have been reported for their capability of producing diverse bioactive compounds which are generally called secondary metabolites. The substances possessing the functions such as defense mechanism and reproduction assistance are classified as secondary metabolites while the compounds directly playing important roles in normal growth and development are primary metabolites such as proteins, carbohydrates, and lipids. Most of the secondary metabolites are of interest to mankind due to their bioactivities, especially cytotoxicity which is one of important defense mechanisms of self-protection (Berdy 2005; Paul *et al.* 2007).

There have been a number of studies reporting that organisms used their own produced secondary metabolites to protect themselves from predators. Penicillin produced by the fungi *Penicillium* spp., one of well-known examples, has shown its antibiotic activity among other surrounded micro-organisms (Berdy 2005). Several secondary metabolites from plants and endophytes have also been investigated for bioactive natural products. Camptothecin which is produced by the plant *Camptotheca acuminata*, shows anticancer activity due to its topoisomerase II inhibitory activity leading to DNA damage and cell death (Wall *et al.* 1966). Currently, bioactive substances from plants, microorganisms and animals have widely been investigated and yet the newly discovered compounds have been introduced to science society. Terrestrial animals might not be a major source of secondary metabolites, however, marine animals, especially with soft bodies, are considered as cytotoxic-agent producers, by reasons of protection from being preyed.

Marine organisms have become a new source for studying new natural products because of their extreme and diverse environment leading to a variety of chemical production. Many new natural products from marine organisms have been constantly discovered. The secondary metabolites from mollusks, sponges, and

tunicates have been reported to usually have cytotoxic and/or antimicrobial activities (Pawlik *et al.* 1988).

Over decades, a large number of cytotoxic agents from marine organisms have been studied. *Bis*-tetrahydroisoquinolinequinone alkaloids, including renieramycins found in the blue sponge *Xestospongia* sp. and jorunnamycins found in the nudibranch *Jorunna funebris* were reported as cytotoxic compounds to human colon carcinoma cell line (HCT116) and human lung carcinoma cell line (QG56) (Charupant *et al.* 2007). Jorunnamycin A (JA), the deangeloyl derivative of renieramycin M (RM), has been recently used as an important starting material to prepare various 22-*O*-acyl derivatives of RENIERAMYCIN M to improve the cytotoxic potency against cancer cell lines as well as to study structure-cytotoxicity relationships (Charupant *et al.* 2009).

To synthesize jorunnamycin A by chemical reaction, the angeloyl group at C-22 of renieramycin M must be removed by cleavage of the ester bond to yield a free alcohol moiety. There is a chemical transformation by the 3-step reactions involving hydrogenation, hydride reduction and oxidation which all required many strong chemical reagents to convert renieramycin M to jorunnamycin A. Interestingly, the normal acid and alkaline hydrolysis reactions were not successful to cleave this ester bond. However, jorunnamycin A was recently isolated in a good yield from the marine nudibranch *Jorunna funebris* pretreated with potassium cyanide (Charupant *et al.* 2009). The gastropod mollusk *J. funebris* is a sponge-feeding animal which specifically feeds on the sponge *Xestospongia* sp. The chemical profile study of the secondary metabolites in this shell-less gastropod was found to accumulate renieramycin M and jorunnamycin A in both visceral and mantle parts while jorunnamycin A was not obtained from the *Xestospongia* sp. sponge. The presence of jorunnamycin A only in the nudibranch leads to a hypothesis that the conversion of renieramycin M to jorunnamycin A is catalyzed by specific enzyme(s) in the nudibranch *J. funebris* tissues. Considering the structures of these two related alkaloids, the enzyme involved in the hydrolysis of the ester bond at C-22 is possibly an esterase type. This study is aimed to detect esterase activity in the crude enzyme extract from *J. funebris* tissues to convert renieramycin M to jorunnamycin A.

Optimization and substrate specificity of the crude enzyme activity will be also studied. The data presented here will be important information as an alternative biotechnology approach to prepare jorunnamycin A.