CHAPTER V

CONCLUSION

The crude enzyme extracted from visceral tissue of *Jorunna funebris*, the nudibranch found in the gulf of Thailand, revealed the obviously detectable esterase activity in converting renieramycin M to jorunnamycin A. The crude enzyme was sequentially precipitated by 55-75% ammonium sulfate. After optimization of the activity under various conditions, it was found that the crude enzyme was active at medium-high temperature (20-50 °C) and tolerant under basic condition, at high pH value (pH 10). Additionally, the crude enzyme maintained the esterase activity at least for four months once stored at -80 °C. This is inferred that the crude enzyme containing esterases for renieramycin M hydrolysis is very robust and stable.

From preliminary screening, the crude enzyme extracted from *J. funebris* catalyzed a specific reaction. The crude enzyme well functioned with the renieramycin M derivative substrates containing aliphatic substituents at C-22 position and the activity was improved when the shorter chain was substituted. This suggests that *J. funebris* may have the evolution to produce the enzyme which is very specific to renieramycin M core structure and particular substitutions. However, there is not yet evidence why *J. funebris* has evolved such a specific enzyme for conversion renieramycin M to jorunnamycin A. There may be a reason for reduction of renieramycin M toxicity, since renieramycin M is the major cytotoxic compound found in their diets, the blue sponges *Xestospongia* sp. Alternatively, *J. funebris* may require jorunnamycin C compound, which is synthesized via jorunnamycin A intermediate and more toxic than renieramycin M and jorunnamycin A (Charupant *et al.* 2007), for chemical defense to protect themselves from predators. Complete purification to isolate and characterize the esterase for renieramycin M hydrolysis is needed for further study.

To summarize, the crude enzyme show the good activity in converting renieramycin M to jorunnamycin A. Thus it is helpful for the production of jorunnamycin A which is used as an intermediate to synthesize renieramycin M derivatives and subsequently study structure-activity relationships of those compounds. This discovery is very beneficial to further investigate many aspects. The preliminary results here will allow a detailed study of protein purification and characterization. Further experiments to complete optimization study will provide more information to make desirable jorunnamycin A production by the enzymatic reaction more possible. Finally, once the renieramycin M hydrolysis enzyme is isolated, the genetic information of its corresponding gene will be retrieved. Thus, the enzyme may be able to be produced in laboratory and sustainable production of jorunnamycin A by the enzymatic reaction will possibly be achieved.