CHAPTER V

CONCLUSION

As part of our continuing investigation on bioactive substances from marine microorganisms, three gram-positive rod-shaped bacteria including the strain Sc004 from seawater and the strains Sc018 and Sc026 from marine sediment around Sichang Island showed interesting biological activities including antiviral activity against *Herpes simplex* viruses type I and type II and antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633. Based on morphological, cultural, physiological and biochemical characteristic studies, the strains Sc018 and Sc026 were identified as *Bacillus*, while the strain Sc004 was still unable to be identified.

Directed by anti-herpes simplex virus activity, fractionation of the butanol extract from the fermentation broth of the gram-positive rod-shaped strain Sc004 yielded the known diketopiperazine, *cyclo*-(L-prolyl-glycyl) (S142 and S151) and the known purine nucleoside, 2'-deoxyadenosine (S147). The known compound, 2'-deoxyadenosine (S147) produced from the culture broth of the marine bacterium Sc004 is the first report of purine nucleosides isolated from a marine microorganism. The bioassay-guided fractionation, using anti-herpes simplex activity of the dichloromethane extract from the culture broth of the *Bacillus* sp. Sc018 yielded three diketopiperazines including two known compounds, *cyclo*-(*trans*-4-hydroxy-L-prolyl-L-phenylalanyl) (F018) and *cyclo*-(D-prolyl-leucyl) (F019), and the new compound, *cyclo*-(D-prolyl-isoleucyl) (F017). The bioassay-directed fractionation, using antibacterial activity against both *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633, of the ethylacetate extract from the fermentation broth

of the *Bacillus* sp. Sc026 led to the isolation of five diketopiperazines including four known compounds, *cyclo*-(L-prolyl-glycyl) (P056WC and P056YEL), *cyclo*-(L-prolyl-D-leucyl) (P049 and P193WC), *cyclo*-(L-prolyl-L-tryptophanyl) (P350), and *cyclo*-(D-prolyl-L-tryptophanyl) (P352) and a new compound, *cyclo*-(D-prolyl-L-phenylalanyl) (P348), together with a new cyclic tetrapeptide, *cyclo*-(4-hydroxy-prolyl-4-hydroxy-prolyl-leucyl-phenylalanyl) (P132). Three macrolactins including the known compound, macrolactin F (P035) along with two new compounds, 7-*O*-succinyl macrolactin F (P129) and 7-*O*-succinyl macrolactin A (P103) were also obtained from the *Bacillus* sp. Sc026.

Eight isolated diketopiperazines and one synthetic diketopiperazine (*cyclo*-(L-prolyl-L-phenylalanyl), dkp27) exhibited new antiviral activity against *Herpes simplex* virus type I and type II, while 2'-deoxyadenosine was inactive antiviral activity against those viruses at a concentration of 50 μg/ml.. Moderate inhibitory activity against HSV-I than HSV-II was noted for *cyclo*-(D-prolyl-leucyl) (F019), *cyclo*-(D-prolyl-isoleucyl) (F017), *cyclo*-(*trans*-4-hydroxy-L-prolyl-L-phenylalanyl) (F018), *cyclo*-(L-prolyl-L-phenylalanyl) (dkp27), and *cyclo*-(L-prolyl-L-tryptophanyl) (P350). The anti-HSV I activity of *cyclo*-(L-prolyl-L-phenylalanyl) (dkp27) and *cyclo*-(L-prolyl-L-tryptophanyl) (P350) was more potent than that of the DL-amino acid containing diketopiperazines due to the folded conformation and the lipophilicity. 2'-Deoxyadenosine (S147) exhibited new antimalarial activity against *Plasmodium falciparum* K1 multidrug resistant strain with ED_{50} at 4.8 μg/ml, while it was inactive against HSV-I and HSV-II. *Cyclo*-(4-hydroxy-prolyl-4-hydroxy-prolyl-leucyl-phenylalanyl) (P132) was a new cyclic tetrapeptide. However, it could not be tested for any biological activities due to the limited amount of sample available for further studies. The isolated macrolactins including macrolactin F (P035), 7-*O*-succinyl macrolactin F (P129), and 7-*O*-succinyl

macrolactin A (P103) exhibited both anti-herpes simplex virus activity and antibacterial activity against *S. aureus* and *B. subtilis* except for 7-*O*-succinyl macrolactin F (P129) showing only antibacterial activity. It indicated that a succinic acid half-ester moiety at C-7 might contribute to the high hydrophilicity and sterically hindrance, which might interfere the transport mechanism through bacterial cell wall and decrease the viral binding to host cells (Vero cells).

This study is the evidence that diketopiperazines, purine nucleosides, macrolactins, and cyclic tetrapeptide are produced by marine bacteria isolated from sediments and seawater. In the last few decades, several diketopiperazines, nucleosides, and macrolides with antibiotic properties have been isolated from marine invertebrates, notably sponges. In all cases, these unique compounds were very minor constituents of the extracts, and this fact, together with the structural characteristics of the compounds, has provided a basis for the hypothesis that such metabolites might actually be produced by microorganisms associated with the invertebrates.