CHAPTER I

INTRODUCTION

The vast number and variety of chemotherapeutic agents such as antibiotics isolated from soil microorganisms have made much contribution to our health during this century. Recently, the probability of discovering novel antibiotics and other bioactive compounds from natural sources is declining as the number of known metabolites increases (Sato *et al.*, 1995). Antibiotic-resistant microorganisms represent an increasingly serious challenge to the successful clinical treatment of human bacterial diseases. A wide variety of approaches to circumventing antibiotic resistance are currently under investigation worldwide. One such approach involves the discovery of new antibiotics belonging to unprecedented chemical structural types that are effective against antibiotic-resistant human pathogens. There, significant challenges, has been done to find new interesting materials, for example, by establishing a mass biological screening program, developing the methods for active metabolite production, and increasing the effort for the isolation of new microorganisms. In line with the above, marine microorganisms have recently attracted worldwide attention as a biomedical resource, since they are thought to possess unequaled potential for metabolite productions. Some chemically interesting and biologicallly significant secondary metabolites produced by marine microorganisms are expected as lead compounds for drug development or pharmacological tools for basic studies on life sciences. Nowadays the discovery rate has reached an astonishing level with many new species being isolated monthly. Based on the finding that ninety percentage of colony-forming bacteria in marine environments are gram-negative (Jensen and Fenical, 1995), but the most actively

bactericidal marine genera were gram-positive *Bacillus* and *Micrococcus* (ZoBell and Rosenfeld, 1947). It seemed resonable to doubt whether many gram-positive bacteria could be identified and be reliably used as a biomedical resource. On the other hand, many marine bacteria are ubiquitous in marine habitats, but it was very difficult to isolate them and control their active metabolite production.

Isolation of microorganisms from marine environment and their cultures in seawater-containing media have provided new substances which could be useful agents. Marine microorganisms are more practical resources of useful agents than high organisms from the marine environment, since an agent produced extracellularly by microbial fermentation is often more easily purified than one produced by and contained in a higher organism. Marine environment may yield strains having new genetic elements such as plasmids; these strains may produce new metabolites with useful new bioactivities (Okami, 1986).

Scrutiny of the scientific literature reveals many references that indicate the presence of bacteria that produce bioactive substances in the marine environment. Three distinct classes of pharmaceutical compounds, antibiotics, antiviral compounds and antitumor drugs, have been associated with marine bacteria. The relationship between genera and ability to produce pharmaceutical compounds is narrow. In particular, antibiotic production has been reported in *Actinomyces*, *Aeromonas*, *Alcaligenes*, *Aliginovibrio*, *Alteromonas*, *Bacillus*, *Chromobacterium*, *Flavobacterium*, *Micrococcus*, *Serratia*, *Streptomyces*, and *Vibrio*. Antiviral activity seems to be restricted to *Flavobacterium*, *Pseudomonas* and *Vibrio*, *Cytophaga-Flavobacterium* (Austin, 1989).

The examples of bioactive substances which were discovered in marine bacteria are as follows:

1. S-228Y, a peri-hydroxyquinone derivative, was isolated from cultures of a marine bacterium, *Chainia purpurogena* SS-228 obtained from shallow sea mud. This compound showed inhibitory activity against gram-positive bacteria except mycobacteria, EHRLICH carcinoma in mice, and dopamine-β-hydroxylase (Okazaki, Kitahara, and Okami, 1975).

2. Aplasmomycin, a polyether macrolide antibiotic, was isolated from cultures of a marine bacterium, *Streptomyces griseus* obtained from shallow sea mud. This compound exhibited inhibitory activity against gram-positive bacteria including mycobacteria (Okami *et al.*, 1976).

3. Istamycins A and B, aminoglycoside antibiotics, were isolated from cultures of a marine bacterium, *Streptomyces tenjimariensis* SS-939. These compounds were found to be as active as fortimicin A and sporaricin A against gram-positive and gram-negative bacteria including aminoglycoside-resistant strains (Hotta *et al.*, 1980).

4. Marinactin, a heteroglycan consisting of glucose, mannose and fucose, was isolated from cultures of a marine bacterium, *Flavobacterium uliginosum* obtained from the homogenates of a sea weed, Ishige foliacea like. This compound exhibited antitumor activity against sarcoma 180 in both solid and ascites tumor in mice (Umezawa *et al.*, 1983).

5. Bisucaberin, a siderophore composed of a cyclic dimer of 1-hydroxy-1,6-diazaundecane-2,5-dione moiety, was isolated from cultures of a marine bacterium, *Alteromonas haloplanktis* SB-1123 obtained from deep-sea mud (Takahashi et al., 1987). This compound showed direct cytostasis for tumor cells but did not cause cytolysis in the absence of macrophages. Cytostatic effect of this compound was attributable to the specific inhibition of DNA synthesis in tumor cells (Kameyama *et al.*, 1987).

6. 3-Amino-3-deoxy-D-glucose, an amino sugar, was discovered from cultures of marine *Bacillus* sp. obtained from deep-sea sediment. This compound exhibited antimicrobial activity by the paper-disk assay against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichai coli*, and *Proteus mirabilis* (Fusetani *et al.*, 1987).

7. Macrolactins A-F, 24-membered polyene macrolactones, have been isolated from cultures of taxonomically unidentified gram-positive bacterium obtained from deep-sea sediment. Macrolactin A showed selective antibacterial activity, inhibited B16-F10 murine melanoma cancer cells *in vitro* assays, exhibited significant inhibition of mammalian *Herpes simplex* viruses (types I and II), and protected T-lymphoblast cells against human HIV viral replication (Gustafson, Roman, and Fenical, 1989).

8. 4-hydroxyphenethyl alcohol (tyrosol) has been isolated from cultures of a taxonomically unidentified gram-positive marine bacterium, symbiosis with American lobster, *Homarus americanus*. This compound showed antifungal activity against crustacean pathogenic fungus, *Lagenidium callinectes* (Gil-Turnes and Fenical, 1991).

9. Caprolactins A-B, caprolactam derivatives, were isolated from cultures of a taxonomically unidentified gram-positive bacterium obtained from deep-ocean sediment. These compounds showed cytotoxic activity against human epidermoid carcinoma (KB) cells and human colorectal adenocarcinoma (LoVo) cells and exhibited antiviral activity against *Herpes simplex* type II virus (Davidson and Schumacher, 1993).

10. Andrimid and moiramides A-C, derivatives of acetates and amino acids, were isolated from cultures of a marine bacterium, *Pseudomonas fluorescens* obtained from tissues of an unidentified tunicate. Andrimid and moiramide B were found to exhibit *in vitro* potent inhibition of methicillin-resistant *Staphylococcus aureus* (Needham *et al.*, 1994).

11. Halichomycin, a macrolide antibiotic, was isolated from cultures of a marine *Streptomyces hygroscopicus*, obtained from a marine fish *Halichoeres bleekeri*. This compound exhibited potent cytotoxicity in the P388 lymphocytic leukemia test system in cell culture (Takahashi *et al.*, 1994).

12. Halobacillin, a cyclic acylpeptide, was isolated from cultures of a marine bacterium, *Bacillus* sp. obtained from deep-sea sediment core. This compound showed moderate human cancer cell cytotoxicity (Trischman, Jensen, and Fenical, 1994).

13. Salinamides A-B, cyclic depsipeptides, were isolated from cultures of a marine bacterium, *Streptomyces* sp. obtained from the surface of the jellyfish *Cassiopeia xamachana*. These compounds exhibited *in vitro* potent inhibition of *Streptococcus pneumoniae* and *Staphylococcus pyrogenes*. More importantly, they also showed potent

topical anti-inflammatory activity using the phorbol ester-induced mouse ear edema assay (Trischman *et al.*, 1994).

14. B-1015, a diazene antibiotic, was isolated from cultures of marine bacterium, *Alcaligenes faecalis* SANK 74291 obtained from a small spiral sea shell *Lunella coronata* Gmelin. This compound was moderately active against both gram-positive and gram-negative bacteria especially against *Bacillus subtilis* and *Escherichia coli*. It was also able to induce spheroplast formation against *Proteus mirabilis* SANK 71873 (Sato *et al.*, 1995).

15. B-1371A and B, peptide antibiotics, have been isolated from cultures of a marine bacterium, *Oceanospirillum linum*, obtained from a seaweed. These compounds were catepsin inhibitors (Sato *et al.*, 1995).

16. B-4607A and C, phenazine antibiotics, were isolated from cultures of marine bacterium, *Vibrio* sp. SANK 73794 obtained from a small shell. These compounds showed a broad spectra against gram-positive and gram-negative bacteria (Sato *et al.*, 1995).

17. γ-Indomycinone, a pluramycin metabolite containing an anthraquinone-γ-pyrone moiety, was isolated from cultures of a marine *Streptomyces* sp., obtained from deep-sea sediment core. This compound exhibited marginally cytotoxic to the human colon cancer cell line HCT116 (Schumacher *et al.*, 1995).

18. Thiomarinols A-C, hybrid antibiotics containing pseudomonic acid analogue and holothin, were isolated from cultures of a marine bacterium *Alteromonas rava* SANK 73390 obtained from seawater. These compounds showed strong inhibitory activity against gram-positive and gram-negative bacteria including methicillin-resistant *Staphylococcus aureus* (Sato *et al.*, 1995).

19. Loloatins A-D, cyclic decapeptides, were isolated from cultures of a tropical marine bacterium possibly within the genus *Bacillus*. These compounds exhibited *in vitro* antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and drug-resistant *Streptococcus pneumoniae* (Gerard *et al.*, 1999).

During the course of biological screening using culture of marine bacteria isolated from several seawater and marine sediment samples collected around Sichang Island (an island in the East coast of the Gulf of Thailand), some crude extracts from culture broths of marine bacteria Sc004, Sc018, and Sc026 showed antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, and anti-herpes simplex virus activity. The results showed that these Thai marine microorganisms may be potential sources of novel bioactive compounds and should be extensively investigated. Therefore, the main objectives of this investigation are as follows:

1. To isolate bioactive compounds from culture broths of marine bacteria by means of bioassay-directed fractionation.
2. To elucidate the chemical structures of the isolated compounds.
3. To evaluate the antibacterial and anti-herpes simplex virus activities of the isolated compounds.

4. To analyse the structure activity relationship (SAR) of the isolated bioactive compounds.