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There is a tremendous level of worldwide interest in marine natural products with therapeutic potential in industry, academia, and government research labs, largely because natural products generally continue to be viewed as one of the few de novo sources of drug discovery, yielding unorthodox and often unexpected chemical structures that offer novel points of departure for molecular modification leading to clinically available drugs (de Souza et al. 1982).
In the United States, most major pharmaceutical companies are actively acquiring terrestrial and marine natural products, extracts from terrestrial and marine macroorganisms and microorganisms, and/or cultures of terrestrial and marine microorganisms for the purpose of finding new, small molecule lead structures. To our knowledge, many of the major pharmaceutical companies in Japan and Europe are involved as well. The U.S. government has provided substantial support since the 1970s for academic research on biologically active marine-derived compounds; government agencies include the National Institutes of Health (NIH)—the National Cancer Institute (NCI) and the National Institute of Allergic and Infectious Diseases (NIAID), as well as the National Sea Grant College Program/National Oceanic and Atmospheric Administration (NOAA) (Persinos 1989c, 1990c, 1991c). Increased interest by the U.S. government in biologically active marine and terrestrial natural products has recently led, for example, to the formation by NIH of National Cooperative Natural Product Drug Discovery Groups, which include most of the prominent academic labs engaged in marine and terrestrial natural products research, several of the major pharmaceutical companies, and representatives from NIH for the purpose of discovering new structural leads with activity against cancers, human immunodeficiency virus (HIV), and opportunistic infections associated with acquired immune deficiency syndrome (AIDS) (Persinos 1990b, 1990c). Strong programs in natural products research have historically existed in Europe and Japan. The tremendous level of interest by Japan in the development of marine resources has recently led their government, industry, and universities to undertake major, long term initiatives in “marine biotechnology,” which include efforts to produce marine-derived pharmaceuticals (Persinos 1989c, 1990a). In fact, two major facilities were recently built expressly for this purpose, one in the city of Kamaishi, to take advantage of organisms found in the cold currents off Japan’s northeast coast, and the other in the city of Shimizu, where the currents are warm.

Current interest in natural products as a source of therapeutic agents is not surprising considering the major role that natural materials and natural products have played in the development of modern therapeutics. Throughout the 5000 years of recorded history of medicines, which includes records from China (Kim 1967), the Middle East, South America, Europe, and Mexico, until the 1800s, the primary source of medicines consisted of terrestrial plants (in the form of herb preparations; “Galenic” medicine) (Burger 1986). Indeed, the development of modern therapeutics is reported to have begun with digitalis, a mixture of steroid (cardiac) glycosides from several plants, including purple foxglove, Digitalis purpurea, which was used in 1785 by a British physician to treat “dropsy,” a condition caused by congestive heart failure and characterized by an accumulation of fluid (Burger 1986). Since the 1800s, numerous naturally occurring compounds have been purified and used for medicinal and health purposes, including plant metabolites, steroid hormones (androgens, estrogens, progestins), glucocorticoids (cortisone), pituitary hormones (antidiuretic hormone (ADH), adrenocorticotropic (ACTH), vasopressin, oxytocin), and vitamins (vitamin D, cod liver oil) (Physicians Desk Reference 1991; Scrip 1991; Guyton 1991).
With the recognition of the microbial basis for infectious diseases in the latter half of the 1800s and the discovery of the “lysis factor” (penicillin) from *Penicillium notatum* in 1928, the stage was eventually set in the 1940s for the use of microorganism-derived natural products as medicines, initially as antibiotics (see discussion in Reuben and Wittcoff 1989). Shortly after the publication of studies on penicillin, an intense search ensued to find new and better microorganism-derived antibiotics (Burger 1986). Near a sewage outfall in the Mediterranean, an antibiotic-producing fungi, *Cephalosporium acremonium*, was isolated (Abraham 1962; Abraham and Newton 1967). The biologically active components were named the cephalosporins, which are structurally related to the penicillins (Reuben and Wittcoff 1989; Caprile 1988), and some would claim are marine-derived (Rinehart 1988a; Torres 1988). The cephalosporins turned out to be significantly more active than the penicillins against Gram-negative microorganisms and are resistant to degradation by staphylococcal β-lactamase (Caprile 1988). The cephalosporins are broadly used therapeutically as well as prophylactically (prior to surgery) (Kaiser 1988). Since 1964, which is when the first member of three generations of cephalosporins was introduced, over 20 cephalosporins have been marketed (Caprile 1988). Numerous additional microorganism-derived compounds are used or are being developed not only as antibiotics, but in most major disease areas (Scrip 1991; Hall 1989). Notable examples are the immunosuppressive agents, cyclosporin (Dreyfuss et al. 1976) and FK506 (Kino et al. 1987a, 1987b), numerous antitumor antibiotics including bleomycin, calicheamicin, esperamicin, mitomycin C, CC-1065, and actinomycin D (Williams et al. 1989 and references therein), and the cholesterol-lowering agent, mevinolin (Alberts et al. 1980).

Toward the end of the 1980s, approximately 75% of the top 20 hospital drugs (in total U.S. dollar sales) were derived from natural sources (mostly antibiotics), and approximately 20% of the top 100 most widely prescribed ethical drugs were also derived from natural sources, i.e., antibiotics from fermentation broths and alkaloids from plants (Reuben and Wittcoff 1989). Microorganism-derived antibiotics are also among the largest volume pharmaceuticals consumed, approximately 15% of the total annual tonnage. (Vitamins and aspirin/acetaminophen comprise most of the remaining tonnage.) Fermentation yields all antibiotics except chloramphenicol and it yields biotechnology-related products as well. In contrast, chemical synthesis and semisynthesis yield the majority of heart drugs, central nervous system agents, antihistamines, analgesics, and anti-inflammatory drugs.

The world’s oceans represent an enormous resource for the discovery of potential therapeutic agents and will ultimately yield as many pharmaceutical leads as have been obtained from the terrestrial biosphere. This prediction is based on the following information.

1. The world’s oceans cover more than 70% of the earth’s surface (Barnes 1980) and contain well over 200,000 invertebrate and algal species (George and George 1979; Winston 1988). There exist nearly 150,000 species (Winston 1988) of algae (seaweed), green (Chlorophyta), red (Rhodophyta),
and brown (Phaeophyta), and some groups of marine invertebrates in which new chemical structures or biological activities have been reported: sponges (Porifera), cnidarians or coelenterates (corals, octocorals (including sea fans), hydroids, and sea anemones), nemertean worms, bryozoans, ascidians (tunicates including sea squirts), molluscs (sea snails and sea slugs), and echinoderms (brittlestars, sea urchins, starfish, and sea cucumbers). All but two of the 28 major animal phyla are represented in aquatic environments; eight phyla, including the Cnidaria, Porifera, Bryozoa, and Echinodermata, are exclusively aquatic, and primarily marine (Barnes 1980).

2. In terms of known species and habitats, and excluding insects, the world's oceans represent our greatest resource of new natural products (Munro et al. 1987). Indeed, since about 1975, approximately 4000 new marine natural products have been reported from a relatively small number of research labs (Faulkner 1984a, 1984b, 1986, 1987, 1988, 1990, 1991). Unfortunately, only a small percentage of these compounds has been fully evaluated for biological activity relevant to humans and only a few have been evaluated in clinically relevant assays. A clinically relevant assay is loosely defined as one in which a positive (potent) response would elicit very serious interest by a (pharmaceutical) company or organization that has sufficient resources and experience to successfully develop a therapeutic agent.

3. Most nonmoving (sessile) marine invertebrates (e.g., Figure 5-1: Porifera-Demospongia, sponges; Coelenterata, sea fans, soft corals, hard corals), and algae (Figure 5-1: Rhodophyta, red algae; Phaeophyta, brown algae; and Chlorophyta, green algae) contain a primitive immune system, as do plants and microorganisms found in the terrestrial biosphere (vide supra). It has been hypothesized (Hall 1989; Williams et al. 1989) that some terrestrial, and, by analogy, some marine organisms that do not contain sophisticated immune systems use natural products, or allelochemicals (Whittaker and Feeny 1971), in response to a variety of ecological, behavioral, and physiological factors (Bakus et al. 1986; Davis et al. 1989; Geiselman and McConnell 1981; McConnell et al. 1982; Hay and Fenical 1988; Hay et al. 1987; Paul 1988; Paul and Fenical 1987; Paul et al. 1987). In order to survive and proliferate in their environments, these primitive organisms must produce compounds that are used to chemically defend themselves and/or to assist in the prevention of overgrowth or fouling. These metabolites also have therapeutic potential against human diseases because of very specific interaction with receptors and enzymes (Hall 1989; Williams et al. 1989). The rationale for this specificity of biological activity is that specialized vertebrate cells such as those of the humoral, nervous, immune, and vascular systems, appeared relatively recently in evolution, while the transmitter molecules they use, such as hormones, neuropeptides, and biological response modifiers as well as allelochemicals, may have arisen much earlier in unicellular or simple multicellular organisms (Gilbert 1977; Nisbet and Porter 1989; Roth et al. 1986). Indeed, it is believed that algae and bacteria have existed up to possibly 3.5 billion years ago, and relatively
FIGURE 5-1 The phylogenetic tree. Reproduced with permission from Scheuer (1973).
simple invertebrates have existed for approximately 0.8 to 1.0 billion years, whereas the first primitive vertebrates did not develop until 500 million years ago, and early mammals have existed only for 180 million years (Tullar 1972).

Because of the rather short period of time (20 years) that a relatively intense effort has been made isolating and identifying new marine-derived natural products, and of the even shorter period of time, perhaps 5 to 10 years, that the majority of the research efforts in this area have clearly shifted from finding new compounds to finding biologically active compounds in clinically relevant assays, it is not surprising that only a handful of compounds has made it to clinical trials or beyond. Among these compounds are bryostatins, didemnin B, manaoalide, the pseudopterosins, and Ara-A and Ara-C, synthetic analogs of marine sponge-derived compounds. Numerous additional marine-derived compounds, however, have been found to be useful biological probes or biochemical tools, or have been studied because of their unusual structures and/or biological activities, including marine-derived prostaglandins, palytoxin, ciguatoxin, brevetoxin, okadaic acid, tetrodotoxin, saxitoxin, calyculins, and kainic acid. Manoalide is also being sold commercially as a biochemical probe.

In this chapter, we hope to show that marine natural products represent a promising source of potential therapeutic agents, to review some of the strategies and approaches by academic and industrial labs that have led to and are leading to the discovery of marine natural products with therapeutic potential, to highlight the sources of biologically active marine natural products and collection techniques of their respective source organisms, to review the history and status of selected marine-derived compounds that are at various points along the discovery and development path, and to present and discuss some of the unique difficulties in the therapeutic development of marine-derived compounds. We have chosen to focus our discussion on relatively low molecular weight compounds derived primarily from marine macroorganisms, such as invertebrates and algae, and from marine microorganisms; we have chosen not to discuss marine-derived biopolymers, i.e., agglutinins, polysaccharides, and glycoproteins (Shimizu and Kamiya 1983), high molecular weight toxins, i.e., conotoxins, sea anemone polypeptide toxins, jellyfish toxins, and marine-derived neurotoxins (Hall and Strichartz 1990), diagnostic reagents from marine organisms, for example, the horseshoe crab (Cohen 1979), and compounds from marine vertebrates. This chapter covers the literature to approximately the middle of 1992.

5.1 SOURCES OF MARINE NATURAL PRODUCTS WITH THERAPEUTIC POTENTIAL

A successful drug discovery program clearly needs continuous sources of new compounds, and a program planning for continued success cannot afford to exhaust its sources. Samples for screening may consist of in-house compound libraries, compounds from various chemical companies and universities, and/
or collections of natural materials. The acquisition of natural materials from the marine environment was slow to develop for various reasons; however, this situation has changed dramatically over the past 10 to 20 years.

5.1.1 Collection of Macroorganisms
The first step in the discovery of marine-derived compounds with therapeutic potential is the collection of marine organisms or marine material from which microorganisms are isolated (Pomponi 1988; Reed and Pomponi 1989). An intensive and systematic collection of marine organisms for compounds with therapeutic potential was hampered in the past by the general lack of (1) familiarity chemists engaged in this research had with the marine environment, (2) lack of documented ethno-natural history of marine organisms for medicinal purposes (Scheuer 1988), (3) appropriate collection tools, techniques, and protocols, and (4) access to clinically relevant assays by those who study marine-derived compounds. These problems have largely been overcome.

Three developments that greatly aided the systematic collection of marine organisms for compounds with therapeutic potential include the advent of scuba about 40 years ago, the development of deep water collection tools over the past 20 years (for example, at Harbor Branch Oceanographic Institution, HBOI), and, probably most importantly, a much closer collaboration among scientists in a variety of disciplines, i.e., marine biologists, ecologists, chemical ecologists, cell biologists, biochemists, pharmacologists, and natural products chemists.

Prior to scuba, shallow water samples could be obtained by wading or snorkeling. Collection by these techniques is limited to a depth of 20 feet (~6 m) or less and is generally inefficient. Scuba, however, allows routine collections to depths of 120 feet (~36.5 m) for 15 minutes of bottom time with no decompression stops. Using mixtures of nitrogen and oxygen (nitrox) or helium and oxygen instead of air allows scuba collections of extended duration and depth (Miller 1979). For example, helium and oxygen mixtures may allow dives to 800 feet (~244 m) or greater, and nitrox mixtures (32% oxygen/68% nitrogen) may double bottom time at depths of 50 to 120 feet (15 to 36.5 m). Mixed gas diving is not routine, however, and only a few sample collectors, mainly professional divers, use it. In general, most marine organisms that have been studied to date have been collected by scuba diving.

Deep water collections have been made by dredging and trawling. These are both cost-effective collection methods if the substratum does not cause damage to or snag the gear. There are several disadvantages to these approaches: it is difficult to photograph the organisms in their habitat; encrusting organisms or organisms that grow in crevices, under ledges or on steep rock faces cannot be easily collected unless the hard substrate that supports the organism is collected as well; dredging and trawling put all collected samples in close contact with each other, therefore, some organisms may chemically contaminate others because of exudations or secretions of various compounds;
and the environmental impact of dredging or trawling can be detrimental because the sampling is nonselective and habitats can be damaged or destroyed.

Deep water collections can also be made by manned and unmanned submersibles or remotely operated vehicles (ROVs). The cost of this form of collection is obviously much higher than dredging or trawling (approximately double or triple); however, the environmental impact of this form of collection is negligible. ROVs are used extensively by the oil industry for survey and repair of oil platforms, and by marine biologists, geologists, and archeologists for survey of marine biota, geologic formations, or artifacts, respectively; however, the technology has not progressed sufficiently to render the ROVs cost effective in marine sample collection (S. Pomponi, personal communication). In contrast, manned submersibles, as exemplified by those at HBOI, which is located on the southeast coast of Florida, have performed very well in the selective and pin-point collection (and recollection) of deep water marine samples for pharmacological evaluation. The HBOI-Johnson-Sea-Link submersibles, for example, can accommodate up to a total of four passengers, and operate at depths of 3000 feet (915 m); they have an array of collection devices and associated sample storage containers to facilitate collection of organisms displaying a wide range of shapes and sizes, and they have various video and 35 mm cameras for purposes of documentation. Using the manned submersibles, the research group in the Division of Biomedical Marine Research (DBMR) at HBOI, for example, has collected samples at 100 to 3000 feet (30 to 915 m) from the Galapagos Islands, various locations in the Caribbean, Bahamas, Gulf of Mexico, and in the eastern Atlantic at the Canary and Madiera islands.

5.1.2 Collection Strategy
Because the collection program in the DBMR at HBOI to collect marine macroorganisms has been considered a very good one in the marine field, the collection strategy will be reviewed briefly. For the first several years of the research effort, beginning in 1984, the strategy was to collect representatives of as many shallow and deep water benthic organisms as could be found. As the research program evolved, the effort began to focus more on shallow and deep water sponges, due to their high incidence of biological activity in a variety of mechanism-based and whole cell assays and high abundance (especially as compared with such organisms as tunicates, some bryozoans, and hydroids). Because of the specialized tools available for collecting of deep water samples, an important niche of this sample collection effort has been deep water sponges. Out of approximately 150 described species from deep water environments in the West Indian biogeographic region (Van Soest and Stentoft 1988), the DBMR/HBOI collection contains nearly all these; further, the collection contains at least another 20 to 40 new, undescribed species of deep water sponges. For comparison, the total number of described shallow
and deep water species from the West Indian biogeographic region is approximately 600 (Pulitzer-Finali 1986).

As a consequence of their rather enormous collection effort, the current DBMR/HBOI collection consists of a total of several thousand deep water and well over 10,000 shallow water macroorganism samples in sufficient quantities for chemical fractionation. To our knowledge, this is the largest marine sample collection in the world. The number of discrete samples (with unique taxonomy) is probably less than one-third, and is an estimate based on the taxonomic identification to the genera level on less than one-quarter of the samples. (Taxonomic identification of these samples has been driven by biological activity of the respective extracts; inactive samples have not been identified unless the taxonomic identification has been trivial.) Other marine research groups have successfully focused their collection and drug discovery efforts on cnidaria (see discussions on the pseudopterosins and the fuscosides), blue green algae (Moore and Patterson 1988; Moore et al. 1988), and marine-derived microorganisms (Gustafson et al. 1989; Kobayashi 1989; Okami 1988).

5.1.3 Marine-Derived Microorganisms
Microorganisms in the terrestrial environment are a proven source of antibiotics and other therapeutic agents. It would seem likely that microorganisms derived from the marine environment should also yield therapeutic agents; however, chemical and biological studies have been slow to develop (Faulkner 1990, 1991). In general, marine-derived microorganisms have been isolated from environmental samples, i.e., sediments, water samples, decaying pieces of wood, etc., or from macroorganisms. To date, new structures have been found in marine-derived bacteria (Burkholder et al. 1966; Gustafson et al. 1989; Takahashi et al. 1987; Umezawa et al. 1983; Wratten et al. 1977), including actinomycetes (Nakamura et al. 1977; Okami et al. 1979; Sato et al. 1978), and in marine-derived fungi (Poch and Gloer 1989a, 1989b).

Isolation techniques for marine-derived microorganisms are relatively straightforward. For example, to obtain microorganisms from macroorganisms, macroorganisms are typically rinsed with sterile seawater, blotted, ground in artificial seawater or filtered seawater from the site, diluted, and plated onto several different agar media (Wilkinson 1978). Several general and several selective media are usually used for primary isolation cultures. For example, general media may include Zobell’s marine agar (Zobell 1941) or peptone-yeast extract-seawater (Weyland 1969); actinomycete-selective media may include starch-yeast, extract-seawater, chitin-seawater, or glyceral-arginine agar-seawater with trace metals and vitamins added (Nonomura and Ohara 1969) as well as cycloheximide to inhibit fungal growth. Agar cultures can be incubated at different temperatures. Cultures are examined periodically and colonies are transferred to appropriate media for purification, identification, and biological and chemical studies.
A controversial facet of marine-derived microorganisms is their putative role with respect to the origin of bioactive natural products from marine macroorganism-microorganisms associations (Ireland et al. 1988). Symbiotic microorganisms have been repeatedly suggested as being the direct or indirect sources of bioactive metabolites in marine sponges (Frincke and Faulkner 1982; McCaffrey and Endean 1985) and other invertebrates, i.e., tunicates (Wright et al. 1990) and bryozoans (Fenical 1991). Symbiotic microorganisms, in the broadest working definition of the term, may be mutualistic or parasitic with the associated host animal and the symbiosis may be obligatory or facultative (Isenberg and Balows 1981). Actinomycetes are ubiquitous in the marine environment (Goodfellow and Haynes 1984; Weyland 1969), and marine invertebrates contain diverse communities of microorganisms (Bergquist 1978; Colwell 1991), including actinomycetes (E. Armstrong, personal communication) and cyanobacteria (Wilkinson 1980). Only several specific examples exist, however, in which secondary metabolites initially extracted and identified from macroorganisms, i.e., sponges, could be produced by fermentation of specific microorganism strains isolated from the respective macroorganism (Stierle et al. 1988; Elyakov et al. 1991). A noteworthy case in regard to marine vertebrates is that of the fish-derived N-3 fatty acids, which seem to have great value for preventing cardiovascular disease (Leaf 1985); they may indeed be produced by associated marine microorganisms (Kitagawa et al. 1989).

5.1.4 Distribution of Biologically Active Marine Natural Products

As evidenced by the numerous reviews of marine natural products (Faulkner 1984a, 1984b, 1986, 1987, 1988, 1990, 1991) and as will be shown in this chapter, the marine environment houses a vast number of new types of chemical structures; many of these structures represent new classes unknown previously from nature or synthesis. Analysis of these reviews indicates that a substantial amount of research has been conducted by chemists in Europe, especially Italy, and in Australia, New Zealand, Israel, Japan, and North America. The greatest number of marine-derived compounds have been isolated from shallow water organisms and their structures published by North American and Japanese researchers, who are located at academic institutions. To our knowledge, a greater number of academic and industrial research groups have become involved in the past several years in the search for biologically active and novel compounds from marine-derived microorganisms, and we expect that this increased effort will be reflected in both the scientific and patent literature in the next several years. Collection sites for the organisms that contain these compounds span the globe and include the Caribbean, Indo-Pacific, Mediterranean, Red Sea, and the Antarctic. Relatively few deep water marine organisms have been investigated, although they appear to be fertile grounds for biologically active compounds. Most shallow
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and deep water macroorganisms have been collected in tropical and subtropical latitudes, presumably because of milder conditions for sample collection, and because of the greater biodiversity of available organisms (Ricklefs 1973). These factors certainly facilitate the collection of a greater number of different organisms per collecting effort, though sample collection in higher latitudes has also been productive.

The earlier reviews by Faulkner show a phyletic distribution of all marine natural products reported between 1977 and 1985 (Ireland et al. 1988). During this time period, most marine natural products were derived from algae > sponges > cnidaria > echinoderms > tunicates > bryozoans > microbes. In part, this trend is related to the relative abundance of the respective organisms; i.e., tunicates, bryozoans, and hydroids are prolific producers of structurally unusual and biologically active natural products. However, these animals are not as abundant as algae, sponges, cnidarians, and echinoderms. With respect to their biosynthetic capabilities of secondary metabolites, algae, sponges, and cnidaria produce a greater proportion of terpenoids than any other compound type; in all three groups of organisms, the second most abundant biosynthetic class of compounds is polyketides. From our own analysis of the compounds reported since 1985, the phyletic distribution of marine natural products is approximately the same. However, the total number of compounds reported from sponges is now about the same as those reported in algae, and there have been significant increases in the number of compounds reported from tunicates and marine-derived microorganisms.

Analyses of reviews that have emphasized biologically active marine natural products (Fusetani 1987; Krebs 1986; Munro et al. 1987) show that virtually all sessile organisms are represented, i.e., sponges, cnidarians, tunicates, bryozoans, hydroids, zoanthids, and micro- and macroalgae. Therefore, although our own experience has led us to focus our efforts on sponges, we believe it is premature to conclude that there may be preferred organisms for active marine-derived compounds for any particular disease state; further, deep water macroorganisms, marine-derived microorganisms from any environment, and organisms from stressed environments, i.e., hydrothermal vents, anaerobic environments, etc., have not been sufficiently studied in any case. The search continues.

5.2 APPROACHES TO THE DISCOVERY OF BIOLOGICALLY ACTIVE MARINE NATURAL PRODUCTS

With few exceptions, intense and systematic bioassay-guided studies of extracts and compounds from marine organisms by academic, government, and industrial research groups using clinically relevant assays to discover naturally occurring substances with therapeutic potential have only been underway for less than 10 years. Because of the tremendous advances in the understanding
of the biology of certain diseases and the concomitant explosion of new assays, researchers are now in a position to explore fully the potential of marine natural products. These biological advances highly complement the development of new chemical technology, i.e., new separation and structure elucidation techniques.

In general, drug discovery strategies can be trivially separated into three categories:

1. Chemically driven, finding biological activities for purified compounds.
2. Biologically driven, bioassay-guided approach beginning with crude extracts.
3. Combination of chemically and biologically driven approaches (vide infra).

For marine-derived drug discovery, strategies may involve one or more of the following elements:

1. In vivo screens
2. Mechanism-based screens
3. Functional, whole cell, or tissue-based assays
4. On-site assays versus post-collection assays
5. "Dereplication," via biological profiles or chemical profiles, e.g., thin layer chromatography (TLC), nuclear magnetic resonance (NMR), and high pressure liquid chromatography (HPLC)

The chemically driven or "traditional grind-and-find" approach (Persinos 1991b) has been pursued vigorously, primarily by academic research groups. In fact, in the 1950s through the 1970s, the majority of the academic effort in studies of marine natural products was "chemistry driven," i.e., the object of the search was novel compounds from marine sources. TLC (for use of TLC in the field, see Norris and Fenical 1985) and \(^1\)H NMR (D.J. Faulkner, personal communication) have been used extensively to "screen" crude extracts and solvent partitions for unusual, and, therefore, interesting patterns. More recently, \(^13\)C NMR has been used (Manes et al. 1985; Crews et al. 1986; Adamczeski et al. 1988). The structural elegance and number of the compounds found in various reviews on marine natural products attest to the success of this approach. It should be noted that isolation and structure elucidation of new, structurally complex compounds have provided immense gratification to marine natural products chemists and, indeed, to academic synthetic chemists who thrive on new synthetic challenges.

With respect to discovering potential therapeutic agents, the chemically driven approach is a viable one. Through a marine pharmacology program at the University of California-Santa Barbara (UCSB) and Scripps Institution of Oceanography (SIO), which has been funded for nearly 20 years by Sea Grant/NOAA, over 1200 pure compounds isolated primarily in the laboratories of W. Fenical (SIO) and D.J. Faulkner (SIO) have been evaluated by
R. Jacobs (UCSB). Although many of these compounds were isolated based on the antibacterial, cytotoxic, and/or anti-inflammatory activities of the respective extracts, many were isolated based on purely chemical, chemotaxonomic, or chemical ecological interests and also screened by R. Jacobs (Jacobs et al. 1985). The anti-inflammatory properties of manoalide (see Section 5.5.3), for example, were discovered through an essentially chemically driven process. In contrast, the anti-inflammatory properties of the pseudopterosins (Section 5.5.4) and the fuscosides (Section 5.4.3) were discovered from a more activity-directed effort. The chemically driven approach has also served other valid scientific purposes, i.e., the structural information can be used to help solve taxonomic problems associated with a variety of marine organisms (Bergquist and Wells 1983; Fenical and Norris 1975; Lawson et al. 1984; Liaaen-Jensen et al. 1982; Pomponi et al. 1991).

Beginning in the 1970s through today, the majority of the academic-based research efforts has become essentially "biologically driven," i.e., the object of the search has shifted to discover marine-derived compounds with biological activity. The biological activities include exploring their potential as agro-chemicals (Crawley 1988) and pharmaceuticals, as well as their possible chemical ecological roles (Bakus et al. 1986; Hay and Fenical 1988). In addition, a few marine-derived compounds have been isolated and their structures elucidated based on "ethno-natural history" (Scheuer 1988); compounds and organisms that have been implicated as public health hazards (Haddad et al. 1983), i.e., tetrodotoxin (Nakanishi et al. 1975), red tide and shellfish toxins (Hall and Strichartz 1990), and ciguatoxin (Yasumoto and Murata 1990) are prime examples.

The screening programs for antitumor agents at NCI have been biologically driven since their inception in 1958 (Suffness et al. 1989). However, the majority of the (U.S.) academic-based marine natural products chemistry groups were not funded by NCI on a grant or contract basis until the 1970s and 1980s. The current screening program utilizes an extensive human tumor panel that includes a number of cell lines which are representative of solid tumors having different tissue origin and clinical responsiveness, as well as murine and human leukemia cell lines. This approach is predicated on the assumption that agents can be identified that exhibit selective activity toward particular tumor histiotypes, and such selectivity is detectable in long-term, tissue-culture-adapted cell lines (Johnson and Hertzberg 1989). Numerous marine-derived, pure compounds have been screened by this panel, and several compounds have current preclinical interest (D. Lednicer, personal communication).

In general and by comparison, drug discovery in industry has "evolved to the use of specific assays with target receptors and enzymes involved in pathogenesis of disease rather than cellular or tissue assays" (Johnson and Hertzberg 1989) and has benefitted immensely from breakthroughs in receptor technology (Hall 1989; Reuben and Wittcoff 1989). These assays reflect new opportunities due to the recent identification of previously unrecognized bio-
molecular targets for therapy (Larson and Fischer 1989). More specifically, this approach for most disease areas is characterized in industry by:

1. Essentially exclusive reliance on biological activity of crude extracts in numerous target-specific assays, i.e., enzyme assays and receptor-binding assays, for selection of crude extracts and bioassay-guided fractionation of the crude extract (prioritization criteria emphasize selectivity and potency).
2. High volume, automated screening, i.e., thousands of samples per year for smaller companies and thousands of samples per week for larger companies.
3. The use of “functional” or whole-cell assays to confirm activity in a particular disease state and to further prioritize samples for fractionation.
4. The use of genetically engineered microorganisms, enzymes, and receptors.

For example, the targeted approach in the antitumor area includes assays for multidrug resistance reversing agents, mitogenic growth factors, protein tyrosine kinase antagonists, topoisomerase inhibitors, DNA intercalators, purine/pyrimidine nucleotide biosynthesis inhibitors, tubulin polymerization inhibitors, signal transduction agonists or antagonists (phospholipase a, phosphatidylinositol kinase, protein kinase c), and assays to find compounds that interfere with the expression or function of oncogene products (Larson and Fischer 1989; Johnson and Hertzburg 1989). One major pharmaceutical company reportedly screens every marine- and terrestrially derived extract against 11 to 12 different assays that have indications in the areas of cancer, AIDS, and cardiovascular and inflammatory diseases (Persinos 1989a). The assays are prioritized, and after one has provided five to six lead structures, the assay is dropped or its priority lowered, and another (perhaps unproven) assay is brought on-line, or the priority of another assay is raised.

In the antitumor area, selectivity, potency, and high volume screening are elements common to both the NCI and industry; however, NCI uses whole cell assays and industry uses primarily mechanism-based assays. Both approaches appear to be valid, or, in the case of NCI, yet to be disproven, and arguments have been put forth to support the use of either approach. Arguments against using mechanism-of-action screens as primary screens are that serendipity and pharmacology are lacking, and useful activities may be missed (Suffness et al. 1989). The arguments for whole-cell systems are that general pharmacokinetic problems are addressed; if a compound can’t cross a membrane, it will test negative; also, if metabolic activation is required for activity, then it has a chance of occurring in whole-cell systems. In some areas such as immune modulation, whole cell assays, e.g., mixed lymphocyte reaction (MLR), mitogen response, and lymphokine production assays, rather than receptor or enzyme assays, continue to be used routinely by academic, government, and industrial research groups.

At least in the antitumor area, arguments against the use of whole cell (cytotoxicity) assays are that (1) they are uneconomical and unproductive
5.2 The Discovery of Biologically Active Marine Natural Products

because of the requirement to discriminate bona fide leads with therapeutic potential from the vast number of known cytotoxic natural products and of the large number of cell lines against which extracts must be tested, and (2) the overwhelming majority of cytotoxic natural products have minimal or no significant activity in certain chemosensitive animal tumor models (Johnson and Hertzberg 1989). In addition, a practical concern often faced by pharmaceutical companies when deciding which compounds to develop is whether or not the mechanism(s)-of-action is (are) known, i.e., if it is not known, vis-à-vis mechanism-based screening efforts, and a substantial effort is required to determine the mode of action, or if it cannot be determined, the compound, in all likelihood, will not be pursued (S. Hecht, personal communication).

Because of the low correlation between cytotoxicity and antitumor activity, a number of programs have utilized in vivo tumor models directly for drug discovery (Johnson and Hertzberg 1989). From 1958 through 1985, NCI used in vivo L1210 and P-388 murine leukemia assays as primary screens (Suffness et al. 1989; Boyd et al. 1988) and was successful primarily in identifying compounds possessing clinical activity against leukemias and lymphomas. Unfortunately, they were not very successful in finding compounds active against slow growing tumors in humans. Further, these in vivo assays were expensive, time consuming, and relatively insensitive (Suffness et al. 1989). Our experience at HBOI in other disease areas has shown that activity in in vitro antiviral assays does not translate well to in vivo activity, e.g., using Herpes simplex. In contrast, reasonable correlations exist between in vitro and in vivo antifungal activity, e.g., using Candida albicans, and between in vitro and in vivo immune suppressive activity, e.g., using the MLR as the in vitro assay and the graft-versus-host reaction (GVHR) or splenomegaly model as the follow up in vivo assay.

Chemically and biologically driven approaches can be combined. In our experience, this combined approach has meant selecting extracts for chemical fractionation based on the biological activity profile of the crude extract. However, instead of using a bioassay-guided approach to purify the compounds responsible for the activity of the extract, NMR and TLC are used to isolate the chemically most interesting substances. Ideally, the structurally unusual or novel compounds are also responsible for the activity of the extract. This approach works well when the active compounds are present in high concentration and the assay turnaround time is longer than a couple of weeks. If the concentration is low, as was the case for the ecteinascidins (Wright et al. 1990), structurally complex isoquinoline alkaloids found at a level of 1 mg compound/1 kg of the tunicate, Ecteinascidia turbinata, the active compounds would have been overlooked completely, had a purely chemical approach been employed. This approach is indeed productive with respect to isolating numerous new compounds, at least some of which usually express some of the activity observed for the crude extract, but is obviously not the best method to identify the most active compounds if they are present in low concentration.
An element of the biologically driven approach that has been used by some research groups is on-site or shipboard assays (Rinehart 1988a, 1988b; Rinehart and Shield 1988). Proponents of on-site bioassays point out that recollections of organisms whose extracts have been found active can be made immediately, organisms whose extracts prove to be inactive during the collecting trip can be subsequently avoided, and chances are improved for observing biological activity in extracts/samples that may undergo degradation by enzymes, air, or heat. The requirements for on-site bioassays are that they do not consume much time; they are simple, rugged, and do not involve pathogenic organisms. Arguments against the use of on-site bioassays include the following:

1. Sample collection is an expensive activity, especially with large research vessels and associated submersibles. Therefore, a more efficient and productive plan is to focus efforts on the collections themselves, and fill ship berths with experts in sample collection and organism identification rather than experts in conducting assays.

2. Because the optimum collection strategy is to collect samples in as many different locales and habitats as possible, the charted course for a collecting trip generally does not involve retracing parts of the route; therefore, it is not practical to count on recollecting larger amounts of a particular organism whose extract has exhibited activity. Further, some assays take several days, and waiting at one collecting site or in the vicinity until assay results are evaluated is a waste of collecting resources.

3. A valid strategy to optimize finding new marine-derived compounds with therapeutic potential is to have the respective extracts evaluated in as many clinically relevant assays as possible and making collection decisions on a few simple (usually unsophisticated), rugged on-site assays imposes an unnecessary constraint.

4. Worrying about sample degradation after taking reasonable precautions, e.g., freezing samples or storing them in solvent before biological evaluation, also imposes unnecessary constraints. In our experience, biological activity in most frozen samples remains intact for up to 9 years; it is simply not worth the effort to concern oneself with a minority of samples that are somewhat labile.

An important element for both chemically and biologically driven approaches is dereplication, which has been defined as the elimination of replicates or repeats of materials already isolated and known in the literature (Suffness et al. 1989; Technical Resources Inc. 1987). In a biologically driven approach, the concept is simply to determine as quickly as possible the chemical nature of the compounds responsible for the biological activity of the crude extract. With this information in hand, informed decisions can be made whether or not to expend precious resources on the (bioassay-guided) isolation and structure elucidation of the active compounds. Numerous databases are
available to assist in dereplication (Technical Resources, Inc. 1987), including those assembled by J. Berdy (Institute for Drug Research, Budapest, Hungary), J. French (Warner Lambert pharmaceutical company), D.J. Faulkner (SIO), NAPRALERT (Natural Products Alert, a literature database assembled by N.R. Farnsworth, University of Illinois, Chicago), Chemical Abstracts, and a database assembled initially by P. Crews (University of California-Santa Cruz; Crews et al. 1991), which has been further embellished by our group in the DBMR/HBOI and other researchers in the marine natural products field.

In our hands, dereplication of marine-derived compounds from macroorganisms has consisted of (1) taxonomy, (2) chemical analysis, and (3) biological profile. Our approach has been to first use the biological activity profile of the extract in order to decide whether or not to continue effort on a sample; if the decision is affirmative, then solvent partitions of the extract are prepared and biological activity profiles are obtained for organic and aqueous layers of solvent partitions. If the activity persists in one or more solvent partitions, the taxonomy of the source organism is ascertained by an HBOI expert in sponge, gorgonian, or algal taxonomy or by collaborators whose taxonomic expertise cover other phyla. Concurrently, NMR spectra and TLC data are obtained for the crude extract and solvent partitions. Based on these data and the biological activity profiles of the crude extracts and solvent partitions, several databases are consulted to better understand the nature of the compounds that may be responsible for the biological activity in the source organism:

1. Our own, which has 9 years of biological activity and taxonomy data about marine organisms.
2. The database assembled by D.J. Faulkner (SIO), which has information retrievable by organism type, name of researcher, compound name, genus or species name, molecular formula, or molecular weight.
3. The database assembled by P. Crews.
4. Chemical Abstracts.

The Crews database can be searched by a combination of structure/substructure, number of carbons and hydrogens in molecule (so-called Attached Proton Test (APT) formula), molecular weight, and taxonomy, if known.

With respect to the various approaches that have been used to isolate marine natural products with therapeutic potential, a somewhat obvious truism can be put forth—one usually finds what one is looking for. If novel compounds are sought (chemically driven approach) (which may subsequently be evaluated for biological activity), NMR, TLC, HPLC, and/or liquid chromatography/mass spectrometry (LC/MS) can be used to “screen” extracts, and taxonomic identification of the respective organism can be used as well to dereplicate. If biologically active compounds are sought (biologically driven approach), mechanism-based, functional whole cell, tissue-based or in vivo
assays are used to screen extracts and guide chemical fractionation. In regard to marine natural products, the biologically driven approach is very appropriate because many compounds whose structures have been published have not been thoroughly evaluated for biological activity. Further, as is well appreciated, a new assay redefines an otherwise exhausted compound and extract library into a whole new sample set. The particulars of a biologically driven approach are critical, however, if marine-derived compounds are sought with therapeutic potential. Indeed, discriminating and predictive assays are required to find compounds that will be used therapeutically not within the next year but rather in 5, 10, or 15 years from now!

To optimize a biologically driven approach, interorganizational collaboration between groups that have expertise in chemistry, biology, research, and development is important, in our view; few academic, government, or industrial research groups truly have the resources, expertise, and interest to be completely autonomous in taking a compound from the ocean to the market. Academic and government research groups typically have some expertise or ready access to expertise in marine biology and ecology, sample collection or procurement, sometimes fermentation, isolation, and structure elucidation or synthesis (usually not both), and biological evaluation in a limited number of disease areas. Many industrial concerns usually have expertise in isolation, structure elucidation, synthesis, biological evaluation, process chemistry, fermentation, preclinical and clinical development, patenting, and marketing. Pharmaceutical companies, however, view natural products as one source in their drug discovery programs, and, therefore, their resources are spread between natural products screening and chemical synthesis.

From our experience, the best relationship to strive for between academic and industrial programs is a scientific collaboration, i.e., one in which extensive exchange of technical information takes place. From an academic point of view, the needs are usually modest, i.e., scientific gratification and monetary support for research. From an industrial point of view, the samples obtained and information exchanged through such a collaboration must ultimately have a commercial benefit (in order to stay in business). Further, a related industrial concern is confidentiality, which, unfortunately, is not the best fit with the academic goal of publishing (or perishing!). A distinct advantage of working with a pharmaceutical company is the possibility that if a marine-derived compound expresses a suitable profile from the company's point of view, resources and expertise to conduct structure-activity optimization studies may become available to study the compound, and to supply sufficient quantities of optimized compound through fermentation or synthesis for preclinical and clinical studies. Typically, academic groups are simply not in a position to accomplish these tasks by themselves; if the goal of a marine-derived drug is to become a reality, some sort of appropriate partnership between an academic group and an interested development partner is necessary and inevitable.
5.3 NOTEWORTHY AND COMMERCIALY AVAILABLE MARINE NATURAL PRODUCTS

In the last 25 years, several marine-derived compounds have generated considerable interest scientifically, commercially, and from a public and health point of view; these include prostaglandins (5.1A, 5.1B, 5.2A, 5.2B, 5.3, 5.4), the potent and structurally complex toxin, palytoxin (5.5), and one of the major causative agents of fish poisoning, ciguatoxin (5.6). Further, because of their unique and potent biological activities, several marine-derived compounds have already found use as biological probes or biochemical tools and are sold commercially, i.e., palytoxin (5.5), brevetoxins (e.g., brevetoxin A, PbTX-1) (5.7), okadaic acid (5.8), tetrodotoxin (5.9), saxitoxin (5.10), calyculin A (5.11), manoolide (5.12), and kainic acid (5.13).

5.3.1 Prostaglandins

Prostaglandins are a group of monocyclic fatty acid metabolites that possess diverse and potent biological activities that include control of blood pressure, renal blood flow, smooth muscle contraction, and gastric acid secretion, and are involved in inflammation (Berkow and Flethcher 1987). In 1969, the Caribbean gorgonian, *Plexaura homomalla* (an animal related to sea fans and sea whips), was reported to contain large concentrations of 15-epi-PGA2 (5.1A) and its diester (5.1B) (Weinheimer and Spraggins 1969). Unfortunately, the 15-epi-configuration rendered these compounds physiologically inactive. However, because of the limited supplies of mammalian prostaglandins from sheep seminal tissues at the time, there was great interest in *P. homomalla* as a potential commercial-scale source of synthetic precursors (Weinheimer and Spraggins 1969; Corey and Matsuda 1987). Further, it was determined that *P. homomalla* grew well at shallow depths in the Caribbean on artificial substrates (Corey and Matsuda 1987). Subsequently, the industrial process chemistry to synthesize active prostaglandins improved such that interest waned in harvesting *P. homomalla* from the ocean.

From a scientific viewpoint, *P. homomalla* continues to generate interest because the prostaglandins found in this animal and a soft coral, i.e., the clavulones (e.g., clavulone I; (5.2A)) found in *Clavularia viridis* (Kikuchi et
al. 1982), are not produced by the mammalian cyclooxygenase/endoperoxide route (Corey et al. 1975); rather, they appear to be produced from arachidonic acid by lipoxygenation at C-8, and subsequent transformation into an allene oxide, followed by oxidopentadienyl cation intermediates. These intermediates readily account for the observed 5,6-cis- and 5,6-trans-prostanoids as well as a cyclopropyl compound that are produced when arachidonic acid is incubated with an acetone powder of *P. homomalla* (Corey and Matsuda 1987; Baertshi et al. 1989).

In addition to the physiologically inactive (15R)-PGA\(_2\), its methyl ester and the clavulones, other marine-derived prostanoids have subsequently been reported from *P. homomalla*, including (physiologically active) (15S)-PGA\(_2\), (15S)-PGE\(_2\), (15S)-5,6-trans-PGA\(_2\), (15S)-13,14-cis-PGA\(_2\), 15-acetate, (15S)-13,14-dihydro-PGA\(_2\) acetate methyl ester, and (15S)-13,14-dihydro-PGA\(_2\) (Bundy et al. 1972; Schneider et al. 1972, 1977a, 1977b), PGF\(_2\alpha\) from the gorgonean *Euplexaura erecta* (Komoda et al. 1979), PGE\(_2\) and PGF\(_2\alpha\) from the red alga *Gracilaria lichenoides* (Gregson et al. 1979), (15S)-PGF\(_2\alpha\) 11-acetate methyl ester and its 18-acetoxy derivative as well the two corresponding free carboxylic acids from a soft coral *Lobophyton depressum* (Carmely et al. 1980), the claviridenones (e.g., claviridenone-a; (5.2B)) from *C. viridis* (Kobayashi et al. 1982), the chlorovulones (e.g., chlorovulone I; (5.3)) from *C. viridis* (Iguchi et al. 1985), the punaglandins (e.g., punaglandin 1; (5.4)) from the octocoral *Telesio riisei* (Baker et al. 1985), and several prostaglandin-1,15-lactones, PGE\(_3\)-1,15-lactone-acetate, PGE\(_2\)-1,15-lactone, and PGE\(_3\)-1,15-lactone, which were isolated for the first time from a natural source, the nudibranch mollusk *Tethys fimbria* (Cimino et al. 1989).
Punaglandin 3, which is the $\Delta^{7,8}$ analog of (5.4) was reported to express cytotoxicity against L1210 with an IC$_{50}$ of 20 ng/mL (Baker et al. 1985); chlorovolune I (5.3) was reported to be cytotoxic against human promyelocytic leukemia (HL-60) cells with an IC$_{50}$ of 0.01 μg/mL (Iguchi et al. 1985); the clavulones showed anti-inflammatory effects at 30 μg/mL in the fertile egg assay, which uses the chorio-allantoic membrane of the chick embryo as the site of induced inflammation (Kikuchi et al. 1982, 1983).

5.3.2 Palytoxin
The independent structure elucidation of palytoxin (5.5) by R. Moore and Y. Hirata and their respective research groups (Moore and Bartolini 1981; Uemura et al. 1981), the subsequent completion of the structure determination of the compound’s stereochemistry, and the synthesis of palytoxin carboxylic acid by Y. Kishi and his research group (Armstrong et al. 1989a, 1989b and references therein), must be considered quintessential scientific
achievements. Palytoxin, a large polyoxygenated metabolite (molecular weight, 2681) obtained from the zoanthid *Palythoa toxica* and other *Palythoa* species, posed an extreme challenge; the structure elucidation was achieved by oxidatively degrading the molecule and elucidating the degradation products, which by themselves are larger than many natural products. From a biological activity viewpoint, it is especially noteworthy because it is the most potent nonproteinaceous toxin known, having a minimum lethal dose in guinea pigs of 0.15 μg/kg (Haddad et al. 1983). (By comparison, the most potent proteinaceous toxin, botulinus toxin A, has a minimum lethal dose of 0.00003 μg/kg.) In the two-stage mouse skin promotion assay, palytoxin is also a potent tumor promoter, although it fails to induce ornithine decarboxylase in mouse skin and does not bind to protein kinase C (Fujicki et al. 1984). Similar to phorbol ester-type tumor promoters, palytoxin has been found to stimulate arachidonic acid metabolism in rat liver cells (Levine and Fujicki 1985). Palytoxin also acts synergistically with phorbol ester-type tumor promoters to promote prostaglandin release (Levine and Fujicki 1985; Levine et al. 1986) and stimulate histamine release from rat peritoneal mast cells (Ouchi et al. 1986). Further, palytoxin has been found to inhibit epidermal growth factor (EGF) binding through a pathway that is not dependent upon protein kinase C (Wattenberg et al. 1987). Palytoxin is commercially available.

5.3.3 Ciguatoxin

Ciguatoxin (5.6) is the primary causative agent of ciguatera, which is a type of food poisoning that occurs intermittently and unpredictably from eating certain coral reef fish (Haddad et al. 1983; Murata et al. 1990; Yasumoto and Murata 1990). The characteristic clinical syndrome includes severe gastrointestinal and neurologic symptoms (Haddad et al. 1983), and affects annually approximately 20,000 people worldwide (Murata et al. 1990). Although the origin of ciguatoxin has been the subject of speculation for centuries (Haddad et al. 1983), the causative organism, the dinoflagellate *Gambierdiscus toxicus*,
was not identified until 1977 (Yasumoto et al. 1977). Ciguatoxin is transferred through the food chain among coral reef biota and accumulates in carnivorous fish, including barracuda, grouper, amberjack, dolphin-fish, pompono, morey eels, and others (Murata et al. 1990).

Although research on the isolation and structure elucidation has been ongoing since the 1960s (Scheuer et al. 1967), the toxin is always present in such trace quantities in affected fish that the structure was finally solved only recently, on less than 1 mg from 125 kg of moray eel viscera (Murata et al. 1990). Using primarily NMR, the structure was determined to be a polyether, structurally similar to some of the other polyether toxins from dinoflagellates, such as brevetoxin A (vide infra).

Numerous marine-derived compounds (in addition to palytoxin) are commercially available. A very brief description of the substance, source, and biological activity or utility for each of these compounds follows.

5.3.4 Brevetoxins A (PbTx-1), (5.7)
These are dinoflagellate toxins (e.g., brevetoxin A, Shimizu et al. 1986), which express toxicity by site-specific binding associated with voltage-sensitive sodium channels, and cause contractile paralysis in animal models (Trainer et al. 1990).

5.3.5 Okadaic Acid (5.8)
This is dinoflagellate toxin (Murata et al. 1982), which was first isolated from a sponge (Tachibana et al. 1981). It has been implicated as a major causative agent of diarrhetic shellfish poisoning. It is a potent tumor promoter, but is not an activator of protein kinase C, and is a potent inhibitor of protein phosphatases-1 and -2A. This toxin expresses marked contractile effect on smooth muscles and heart muscles (Haystead et al. 1989).
5.3.6 Tetrodotoxin (5.9)
This bacterial metabolite (Tamplin 1990) is also found in fish (pufferfish), starfish, crab, octopus, frog, newt, salamander, goby, gastropod, mollusk, flatworm, annelid, zooplankton, and algae (Nakanishi et al. 1975). It is a potent toxin that reversibly blocks sodium channels but has no effect on potassium channel blockage. It is useful as a marker of sodium channels in excitable tissues and can be used to block sodium channels in multiple conducting systems, such as the study of the calcium channel in cardiac muscle (Hu and Kao 1985).

5.3.7 Saxitoxin (5.10)
This bacterial metabolite/dinoflagellate toxin (Hall et al. 1990; Tamplin 1990) is one of the main causative agents of paralytic shellfish poisoning (Hall et al. 1990). It is a potent, reversible channel blocking agent and similar in mechanism-of-action to tetrodotoxin (Kao 1966; Hall et al. 1990).
5.3.8 Calyculin A (5.11)
This sponge metabolite (Kato et al. 1986; Matsunaga and Fusetani 1991) inhibits protein phosphatases A and 2A (which also serve as okadaic acid receptors), promotes tumor growth on CD-1 mouse skin, and induces ornithine decarboxylase in mouse skin (Suganuma et al. 1990; Matsunaga et al. 1991). It also exhibits antitumor activity against Ehrlich and P-388 leukemia in mice (Kato et al. 1986, 1988).

5.3.9 Manoalide (5.12)
This compound is a sponge metabolite that inhibits phospholipase A$_2$ and C and intervenes in the arachidonic acid cascade (Wheeler et al. 1987). (See Section 5.5.4.)

5.3.10 Kainic Acid (5.13)
This red algal metabolite is a well-known anthelmintic (Fattorusso and Piatelli 1980). It is an excitatory amino acid that affects mammalian and amphibian central nervous systems and is used as a neurobiological tool in the study of Huntington's disease and epilepsy (McGeer et al. 1978).

By serving as research tools, these marine-derived compounds have an important spin-off effect of facilitating the discovery of therapeutic agents.
An example of this role can be found in the case of marine neurotoxins such as tetrodotoxin and saxitoxin. These compounds, each of which has a specific and distinct action on the function of the voltage-sensitive sodium channel of excitable tissues, have for years served as probes to investigate the structure and function of the sodium channel protein itself and its role in the overall function of neuromuscular tissue. Such studies lead to the identification of new biochemical targets for chemical intervention and thus facilitate new drug discovery.

5.4 SELECTED COMPOUNDS WITH THERAPEUTIC POTENTIAL

Few marine-derived compounds have been studied biologically beyond a simple assessment of cytotoxicity and antimicrobial activity; therefore, it has been difficult to fully assess their therapeutic potential. The purpose of this section is to share the history and results of in-depth biological studies of several marine-derived compounds that have therapeutic potential: dercitin (5.14), which expresses antitumor activity; mycalamides A (5.26A) and B (5.26B) and the structurally related compound, onnamide (5.28), which also exhibits antitumor activity as well as antiviral activity; discodermolide (5.19) and microcolins A (5.25A) and B (5.25B), which display promising immunosuppressive activity; and fuscoside A (5.23), which is a promising anti-inflammatory agent. These compounds were derived from a variety of sessile, shallow and deep water marine organisms: sponges, gorgonians, and blue green algae, that were collected throughout the world. All these compounds have been or can be synthesized.

5.4.1 Dercitin

Dercitin (5.14) is one of many acridine heterocycles isolated and identified in the last 5 to 10 years from ascidians collected in the Indo-Pacific and Red Sea and shallow and deep water sponges collected in the Indo-Pacific and Caribbean. Dercitin itself was isolated from a deep water Caribbean sponge, *Dercitus* sp. (Gunawardana et al. 1988). Structurally related compounds include nordercitin (5.15A), dercitamine (5.15B), dercitamide (kuanoniamine C; Carroll and Scheuer 1991) (5.15C), cyclodercitin (5.16), dehydrocyclodercitin (5.17), and stellettamine (5.18) (Gunawardana et al. 1989, 1992).
5.4 Selected Compounds with Therapeutic Potential

(5.14) $\text{R} = \text{NMe}_2$

(5.15) $\text{A R} = \text{NMe}_2$

$\text{B R} = \text{NHMe}$

$\text{C R} = \text{NHCOEt}$

(5.16)

(5.17)

(5.18) $\text{NMe}_2$
Dercitin was isolated using a bioassay-guided approach. The *Dercitus* sp. sponge was selected as an antitumor lead based on the in vitro and in vivo P-388 activity of the crude extract, i.e., 100% inhibition of in vitro cell proliferation at 20 μg/mL, and percent treatment control (%T/C) = 140 at 100 mg/kg (intraperitoneal, i.p., QD1-9). Purification of dercitin was achieved by following in vitro P-388 activity.

The concentration of dercitin in *Dercitus* sp. is high, approximately 1% wet weight, i.e., 1 g of compound can be obtained from 100 g of (frozen) sponge. However, the sponge is difficult to collect (S. Pomponi, personal communication); the sponge is encrusting, which means that it requires substantial effort to scrape off the rocks on which it resides, and it is deep water, which means that it can only be collected by submersible. It is not possible to collect this encrusting sponge by trawling or dredging.

The in vitro biological profile of dercitin is found in Table 5-1 (Gunawardana et al. 1988; Burres et al. 1989; S. Cross and P. McCarthy, unpublished data): dercitin (5.14) is 10 to 100 times more active in vitro against P-388 than the congeners (5.15A–5.18) nordercitin (5.15A) IC₅₀ = 4.8 μM, dercitamine (5.15B) IC₅₀ = 26.7 μM, dercitamide (5.15C) IC₅₀ = 12.0 μM, cyclodercitin (5.16) IC₅₀ = 1.9 μM, dehydrocyclodercitin (5.17) IC₅₀ = 9.9 μM, and stelletamine (5.18) IC₅₀ > 60 μM.

The antitumor properties and mechanism-of-action of dercitin have been studied (Burres et al. 1989); dercitin prolonged the life of mice-bearing ascites P-388 tumors (%T/C = 170, 5 mg/kg, i.p., QD1-9), was active against (i.p.) B16 melanoma (%T/C = 125, 1.25 mg/kg, i.p. QD1-9), and modestly inhibited the growth of (subcutaneous, s.c.) Lewis lung carcinoma (median tumor volume/untreated tumor volume = 0.49, 1.25 mg/kg, i.p. QD1-9).

The fused-ring acridine structure of dercitin suggested the possibility of intercalation (Burres et al. 1989). Exogenous (calf thymus) DNA (<1 mg/mL) was found to protect P-388 cells when exposed to dercitin at concentrations <1 μM. From incorporation studies, dercitin disrupted DNA and RNA
synthesis, whereas effects on protein synthesis were less pronounced; this profile is similar to the known intercalators actinomycin D and daunomycin. Further, the addition of either (calf thymus) DNA or (calf liver) RNA to solutions of dercitin altered the visible spectrum of dercitin in a manner consistent with binding, and equilibrium dialysis experiments revealed that dercitin binding to calf thymus DNA was saturable with an affinity of 3.1 μM and maximal binding of 0.2 mol dercitin for each base pair. Dercitin was found to relax covalently closed supercoiled Φx174 DNA with a concentration of 35 nM required for half-maximal relaxation; relaxation was reversible. DNA intercalation by dercitin was not found to be related to affects on topoisomerase II. Dercitin completely inhibited DNA polymerase I/DNase nick translation at 1 μM; however, the effects of dercitin on enzyme activity appeared to be secondary to changes in DNA conformation.

5.4.2 Discodermolide
Discodermolide (5.19) is a new polyhydroxylated lactone from a shallow water Caribbean sponge, *Discodermia dissoluta*, that expresses immune suppressive activity (Gunasekera et al. 1990). The sponges, *Discodermia* species, have been collected in the Caribbean and in waters adjacent to Okinawa, and have been proven to be prolific producers of structurally diverse, biologically active compounds, including the discodermins (Matsunaga et al. 1985a, 1985b), which are cyclic polypeptides containing 13 residues, the calyculins (e.g., calyculin A (5.11) Section 5.3.8), discodermide (5.20) (Gunasekera et al. 1991), polydiscamide (5.21) (Gulavita et al. 1992), and discodermindole (5.22) (Sun and Sakemi 1991). The discodermins are cytotoxic and exhibit antimicrobial activity; the calyculins are potent cytotoxins and were recently found to be protein phosphatase inhibitors; discodermide expresses antifungal ac-
tivity and is related structurally to the antimicrobial agent ikarugamycin, which was isolated from *Streptomyces phaeochromogenes* var. *ikaruganensis* Sakai (Ito and Hirata 1977; Jomon et al. 1972); polydiscamide exhibits in vitro cytotoxicity against P-388 murine leukemia and A549 human lung cancer (Gulavita et al. 1992); discodermindole also expresses cytotoxicity.

The structural types and diversity of the *Discodermia* metabolites argue for some involvement of microorganisms associated with these sponges. Efforts are underway at HBOI to isolate microorganisms that produce these or related compounds (Armstrong et al. 1991).

*D. dissoluta* was selected as a lead based initially on the antifungal properties of a crude alcohol extract against *C. albicans*. The crude extract also expressed cytotoxicity against P-388 and modest immune suppressive activity; however, the cytotoxic properties relegated the extract as a lower priority immunosuppressive lead. Although in vitro P-388 and *C. albicans* activities were followed, fractions were also tested in the MLR assay. The cytotoxic fractions also exhibited potent immunosuppressive activity, and ultimately discodermolide was isolated from these fractions. Consequently, discodermolide was found to inhibit the in vitro proliferation of cultured murine
Discodermide was found to be responsible for the antifungal activity of the *D. dissoluta* extract: *C. albicans* minimum inhibitory concentration (MIC) = 12.5 μg/mL. Polydiscamide was isolated from a different, possibly new deep water species of *Discodermia*, and was isolated simply because it is in high concentration in the water partition of water/butanol solvent partition of an ethanol extract. Its biological activities were subsequently discovered: P-388 IC$_{50}$ = 0.9 μg/mL, A549 (human lung cancer) IC$_{50}$ = 0.7 μg/mL. Discodermindole was isolated from *D. polydiscus* and expressed cytotoxicity: P-388 IC$_{50}$ = 1.8 μg/mL; A549 IC$_{50}$ = 4.6 μg/mL; HT29 (human colon cancer) IC$_{50}$ = 12.2 μg/mL.

Further quantitative studies with discodermolide (Longley et al. 1991a) revealed potent suppression of the murine two-way MLR and concanavalin A (Con A) stimulation of splenocyte cultures, with IC$_{50}$ values of 0.24 and 0.19 μM, respectively. These values are approximately 8- to 20-fold less potent than those that are commonly reported for suppression of murine MLR responses by the immunosuppressive standard, cyclosporine A (CsA). There was no evidence of cytotoxicity for murine splenocytes at discodermolide concentrations as high as 1.26 μM. Discodermolide was also active in suppressing the two-way MLR of human peripheral blood lymphocytes (PBL) (IC$_{50}$ = 5.7 μM), of Con A (IC$_{50}$ = 28.0 μM), and phytohemagglutinin (PHA) (IC$_{50}$ = 30.1 μM)-stimulated PBL. Discodermolide was not cytotoxic to human PBL at concentrations as high as 80.6 μM. In a comparison with the standard immunosuppressive agent, CsA, discodermolide was equally effective in inhibiting T-cell receptor (TCR) and accessory cell independent, calcium dependent PMA-ionomycin induced proliferation of purified murine T-lymphocytes, with IC$_{50}$ values of 9.0 and 14.0 nM for discodermolide and CsA. These findings suggest that discodermolide's suppressive activity reaches beyond T-cell activation via an inhibition of TCR mediated activation or interference with cell-to-cell interaction of B-cells or macrophages. Discodermolide did not significantly suppress the production of IL-2 in PMA-ionomycin stimulated murine splenocytes; however, the expression of the p55 associated IL-2 receptor as measured by fluorescence microscopy and flow cytometric analyses, using 7D4 antibody in these same cells was reduced. Similar results were obtained with PHA-stimulated blast cells obtained from human PBL in which the expression of p55 "Tac antigen" (human IL-2 receptor) was evaluated. The p55 receptor in mouse and human normally has low affinity for IL-2 binding (Malek et al. 1986; Waldman 1986). However, this chain associates with an additional cell surface, a 75 kDa (p75) protein to form an IL-2 receptor of high affinity. Before this apparently unique mechanism of action can be ascribed to discodermolide, it would be of great
interest to determine if such suppression extends to the high affinity, p55-p75 complex.

Additionally, whereas discodermolide suppressed the PMA-ionomycin stimulation of murine T-cells, when examined microscopically, these cells appeared to have already undergone blast transformation, indicating that activation had already taken place (Longley et al. 1991a). Discodermolide’s mechanism of action may resemble that of rapamycin rather than FK506 or CsA (Metcalf and Richards 1990) in that the blockage may occur at G2 rather than at the G0\(\rightarrow\)G1 interface, as for FK506 or CsA. Finally, discodermolide appears not to inhibit protein synthesis per se as indicated by \(^{35}\)S methionine incorporation studies (R.E. Longley, unpublished observations). The precise in vitro mechanism of action of discodermolide still remains to be elucidated.

The in vivo activity of discodermolide has also been studied (Longley et al. 1991b). The compound was evaluated in the GVHR splenomegaly assay, using the BALB/c \(\rightarrow\) CB6\(F_1\) (BALB/c \(\times\) C57BL/6J)\(F_1\) model. In initial experiments, discodermolide was effective suppressing the GVHR in allogeneic grafted mice at 5.0, 2.5, and 1.25 mg/kg when administered as daily, i.p. injections for 7 days. While the two higher dosages resulted in the greatest suppression of the response (219\% and 150\%, respectively), these dosages were associated with a degree of morbidity in each group (two out of five and four out of five survivors, respectively). However, the lower dosage groups (1.25 mg/kg) continued to exhibit substantial suppression of their response (93\%), but with no associated morbidity. Mice similarly treated with 150 mg/kg of CsA exhibited 80\% suppression of the splenomegaly response. In order to determine an endpoint for the in vivo immunosuppressive action of discodermolide, a second dose-ranging experiment was initiated in which mice were treated with 2.5, 1.25, 0.312, and 0.156 mg/kg. Discodermolide suppressed the GVHR at all dosages tested with some morbidity once again associated with the 2.5 mg/kg group. However, all remaining groups exhibited suppression of the response, with the 0.156 group continuing to express 76\% suppression. As a comparison, CsA-treated allogeneic grafted mice demonstrated 90\% suppression. An endpoint for this suppression by discodermolide has yet to be determined.

Splenocytes obtained from discodermolide-treated, allogeneic grafted mice were suppressed in their response to Con A and in their ability to lyse YAC-1 tumor cells in natural killer cell assays compared with their vehicle-treated, allogeneic grafted controls (Longley et al. 1991b). Production of Con A-induced-IL-2 was suppressed only in the highest discodermolide dosage group (5.0 mg/kg), in contrast to suppression of IL-2 by CsA. Discodermolide was also evaluated for its in vivo effect on the primary serum antibody response to the T-dependent antigen, sheep red blood cells (SRBC). Mice were treated with daily i.p. injections of 5.0 mg/kg discodermolide and 150 mg/kg of CsA as a comparative standard. The rather surprising result was that discodermolide did not suppress the SRBC antibody response, even at the highest dosage. By contrast, CsA-treated mice were suppressed in their ability
to mount such a response, a result consistent with the known mechanism of action of CsA. This result indicates that the immunosuppressive effect of discodermolide appears not to be of a general nature and, in fact, seems to be unique.

These results collectively indicate that the in vivo mechanism of action of discodermolide is very different from that of CsA and, in fact, may indicate an entirely novel mechanism of its immunosuppressive effects. An endpoint for suppression of the in vivo GVHR has yet to be reached, however, a conservative estimate of discodermolide's in vivo comparison with CsA (via i.p. routes) indicates that the compound is from 100 to 1000 times more potent in vivo compared with CsA. Further work regarding discodermolide's apparent unique in vivo mechanism of action and evaluation of additional in vivo models of graft rejection are currently underway.

5.4.3 Fuscosides A and B

Fuscosides A (5.23) and B (5.24) are diterpenoid arabinose glycosides, which were isolated from the Caribbean gorgonian Eunicea fusca (Shin and Fenical 1991) through a collaboration between Sea Grant/NOAA-funded research groups at SIO and UCSB. Fuscoside A (5.23), one of the major components of the extract, was found to reduce phorbol ester (PMA)-induced mouse ear edema comparable to indomethacin, whereas fuscoside B (5.24) is slightly less active (Jacobson and Jacobs 1991). However, fuscoside B appears to selectively inhibit leukotriene synthesis (i.e., blocks the production of LTC₄ in murine macrophages activated by a calcium ionophore), but has no effect on the production of PGE₂. The anti-inflammatory effects of the fuscosides are long lasting and have been found to be active in human neutrophil studies as well (Jacobsen and Jacobs 1991).
5.4.4 Microcolins A and B

Microcolins A (5.25A) and B (5.25B) are lipopeptides from the filamentous blue green algae *Lyngbya majuscula* (*Microcoleus lyngbyaceous*) that exhibit potent immunosuppressive activity (Koehn et al. 1992). Samples containing the compounds (5.25A and 5.25B) were collected in the southern Caribbean, off of the coast of Venezuela; samples recently collected near Miami also contain the same suite of lipopeptide metabolites. *Lyngbya majuscula* is notable in that it appears to exist in multiple, chemically distinct, varieties. For example, one or more shallow water strains cause “swimmers itch,” a contact dermatitis caused by the lyngbyatoxins (Cardellina et al. 1979; Aimi et al. 1990) or aplysiatoxins (Moore et al. 1984). Shallow water samples of *L. majuscula* also contain the epimeric lipopeptides, majusculamides A and B (Marner et al. 1977), whereas the deep water variety contains antifungal cyclic depsipeptides, majusculamide C (Carter et al. 1984) and normajusculamide C (Mynderse et al. 1988). Deep water samples of *L. majuscula* have also furnished two cytotoxic lipopeptides, majusculamide D (5.25C) and deoxy-majusculamide D (5.25D) (Moore and Entzeroth 1988), which share structural similarities to microcolins A and B, since they contain an N,O-dimethyl tyrosine instead of an N-methyl leucine.

A crude ethanol extract of the algae showed in vitro activity against murine P-388 leukemia with an IC$_{50}$ of 0.4 μg/mL and immunosuppressive
activity in the MLR; however, the extract expressed lymphotoxicity in the lymphocyte viability assay (LCV) (Koehn et al. 1992). Because of the lymphotoxicity, bioassay-guided purification was undertaken using cytotoxicity as the primary indicator. Later, semipure chromatographic fractions containing microcols A and B were subsequently evaluated in the MLR and LCV assays at nontoxic doses and their impressive immunosuppressive properties were recognized. The in vitro biological profiles of the compounds are presented in Table 5–2.

In an experiment whereby purified T-lymphocytes were induced to proliferate by the combined action of the calcium ionophore, ionomycin, and the phorbol ester, 12-myristate 13-acetate (PMA), microcolin A yielded an EC$_{50}$ value of 7.4 nM; cyclosporin yielded an EC$_{50}$ of 37.5 nM in the same experiment. This experimental result suggested that the apparent mechanism of action of the microcols may not directly involve the T-lymphocyte in relation to its suppressive action in the MLR experiment (Longley and Koehn 1991). Further studies on the effect of microcolin A on IL-2 production and IL-2 receptor expression of Con A-stimulated splenocytes indicate that the mechanism of microcolin A suppressive action does not involve the modulation of IL-2 production or IL-2 receptor expression per se.

From several in vivo experiments (Longley et al. 1993) using Simonsen’s GVHR splenomegaly model, microcolin A at 1.0 mg/kg suppressed the splenomegaly response. A higher dosage regimen (5.0 mg/kg) resulted in death of the animals. Doses lower than 1.0 mg/kg were not associated with suppression of the splenic response. However, suppressed responses of splenocytes

| TABLE 5–2  Biological Activity of Microcols A (5.25A) and B (5.25B)$^1$ |
|---------------------------------|-----|-----|
| **Immunomodulation**            |     |     |
| MLR (two-way) EC$_{50}$ (nM)    | 1.5 | 42.7 |
| TC$_{50}$ (nM)                  | 22.6| 191  |
| Con A mitogen assay EC$_{50}$ (nM) | 1.7 | —    |
| LPS mitogen assay EC$_{50}$ (nM) | 1.9 | —    |
| PHA mitogen assay EC$_{50}$ (nM) | 1.1 | —    |
| **Cytotoxicity**                |     |     |
| P-388 IC$_{50}$ (nM)            | 1.3 | 123  |
| **Antiviral activity**          |     |     |
| HSV-1 ED$_{50}$ (ng/mL)         | 2.5 | 146  |
| TD$_{50}$ (ng/mL)               | 480 | 1185 |

$^1$ Activity for microcols A and B was not detected against the following pathogens: A549 mouse corona virus, PR8-influenza virus, feline leukemia virus, E. coli, B. subtilis, A. nidulans, and C. albicans.
that were obtained from these two lower dosage groups (0.2 and 0.04 mg/kg) were observed for PHA but not for Con A stimulation, indicating a specific cell subset (possibly B-cells or macrophages) that is affected in vivo.

5.4.5 Mycalamides A and B, and Onnamide
Mycalamides A (5.26A) and B (5.26B) are polyoxygenated compounds from the New Zealand shallow water sponge, *Mycale* sp. (Perry et al. 1988, 1990), that exhibit various types of biological activity, including in vivo antitumor activity in a wide variety of tumor models (Burren and Clement 1989) and antiviral activity. The sponge, *Mycale* sp. was collected by the research group at the University of Canterbury during a collaborative effort between this group and the research group at HBOI. The crude sponge extract exhibited potent cytotoxicity and in vitro antiviral activity in assays conducted at the University of Canterbury. Using a bioassay-guided approach, mycalamides A and B were purified and shown to be responsible for the biological activity of the extract (Perry et al. 1988).

Interestingly, the mycalamides resemble pederin (5.27) and related compounds, which were isolated from the terrestrial beetle *Paederus fuscipes* (see references in Perry et al. 1988).
From a concurrent independent investigation of the shallow water Okinawan sponge, *Theonella* sp., at Harbor Branch Oceanographic Institution by the research group from the University of the Ryukus (Sakemi et al. 1988), the structurally related compound, onnamide (5.28) was isolated using a combined chemically and biologically driven approach.

The mycalamides and onnamide express potent cytotoxicity (Burres and Clement 1989), as shown in Table 5–3. All three compounds are highly cytotoxic toward P-388. However, compared with mycalamides A and B, onnamide was found to be less cytotoxic against the HL-60 human promyelocytic leukemia cell line, HT-29 human colon cancer cell line, and the A549 human lung cancer cell line. None of the compounds induced HL-60 cell differentiation (Burres and Clement 1989).

Mycalamides A and B were found to exhibit similar in vivo efficacy in the i.p. P-388 murine leukemia model (Burres and Clement 1989); the best responses for mycalamides A and B were T/C = 140% (10 μg/kg) and 150% (2.5 μg/kg), respectively, whereas onnamide was found to be virtually inactive (best response, T/C = 115% (40 μg/kg)), presumably because of the ion-

|          | Mycalamide A | Mycalamide B | Onnamide |
|----------|--------------|--------------|----------|
| P-388 IC₅₀ (nM) | 5.2          | 1.3          | 2.4      |
| HL-60 IC₅₀ (nM) | 3.0          | 1.5          | 25       |
| HT-29 IC₅₀ (nM) | 2.8          | 1.5          | 180      |
| A549 IC₅₀ (nM)  | 3.6          | 0.6          | 170      |

Adapted with permission from Burres and Clement (1989).

1 P-388, murine leukemia; HL-60, human leukemia; HT-29, human colon cancer; A549, human lung cancer.
izable arginine group in the molecule, which may result in poor uptake/passage across membranes. All three compounds expressed efficacy in the i.p. M5976 reticulum cell sarcoma model (Burres and Clement 1989). The best responses for mycalamides A and B, and for onnamide were T/C = 233% (60 μg/kg, i.p., Q4Dx3), 218% (30 μg/kg, i.p., Q4Dx3) and 183% (20 μg/kg, i.p., QD1-9), respectively. From further in vivo evaluation, mycalamide A (5.26A) has been found to be substantially more active against solid tumors of murine and human origin than against P-388 murine leukemia (Burres and Clement 1989) (Table 5-4).

From experiments aimed toward elucidating the cytotoxic mechanism(s) of action of mycalamides A and B and onnamide, all three compounds were found to markedly inhibit protein and DNA synthesis in P-388 cells, with intermediate effects on RNA synthesis (Burres and Clement 1989). Mycalamide B was found to be the most potent protein synthesis inhibitor and onnamide the least potent. Further, all three compounds were found to inhibit translation in cell-free extracts, using Brome mosaic virus as an RNA substrate in lysed rabbit reticulocytes; mycalamide B was found to be most potent and mycalamide A was found to be least potent.

In experiments to determine whether mycalamide A intercalated into DNA, mycalamide A was found to alter neither the electrophoretic mobility of supercoiled DNA nor lose its antiproliferative effects when combined with thymidine, deoxycytidine, deoxyadenosine, and deoxyguanosine (Burres and Clement 1989).

| Tumor Type            | Site | Schedule | Best Response | Dose (μg/kg) |
|-----------------------|------|----------|---------------|--------------|
| P-388 leukemia        | i.p. | QD1-9    | 56% ILS¹      | 10.0         |
| B16 melanoma          | i.p. | Q4Dx9    | 145% ILS (40)² | 30.0         |
| M5076 reticulum cell  | i.p. | Q4Dx3    | 133% ILS (40)² | 60.0         |
| Colon 26 carcinoma    | i.p. | Q4Dx3    | 49% ILS (20)   | 60.0         |
| B16 melanoma          | i.p. | Q4Dx9    | 43% ILS       | 7.5          |
| B16 melanoma          | s.c. | Q4Dx9    | 0.77 T/C TWI³  | 3.8          |
| Lewis lung             | s.c. | QD1-9    | 0.15 T/C TWI   | 20.0         |
| M5076 reticulum cell  | s.c. | Q4Dx3 (i.v.) | 0.15 T/C TWI | 120.0        |
| MX-1 mammary xenograft| s.c. | Q4Dx3    | 0.21 T/C TWI   | 80.0         |
| CX-1 colon xenograft  | s.c. | Q4Dx3    | 0.29 T/C TWI   | 60.0         |
| LX-1 lung xenograft   | s.c. | Q4Dx3    | 0.76 T/C TWI   | 7.5          |
| Burkitts lymphoma     | s.c. | Q4Dx3    | 0.19 T/C TWI   | 20.0         |

Adapted with permission from Burres and Clement (1989).

¹ Efficacy expressed as increased life span (ILS) of treated mice compared with untreated tumor-bearing mice (>25% ILS = significant antitumor effect).
² Numbers in parentheses indicate percentage of cure rate for treated group.
³ Tumor volume calculated as \((L \times W^2)/2\) and expressed as median tumor weight index (TWI), which represents the ratio of tumor volumes of treated/control tumors.
TABLE 5-5 Antiviral Activity of Mycalamide A (5.26A)

| Virus Type                  | IC_{50} (in ng/ml) |
|-----------------------------|-------------------|
| Herpes simplex Type 1       | 2                 |
| Vesicular stomatitis        | 2                 |
| Polio vaccine Type 1         | 2                 |
| Coronavirus A59             | 10                |
| Human immunodeficiency virus | 6/16              |

In vivo—Murine Coronavirus A59:

- Mycalamide A\(^2\) (5.26A) QD1-9, i.p., 100 μg/kg 100% Survivors, day 14
- Ribavirin QD1-9, i.p., 100 mg/kg 100% Survivors, day 14
- Control 0% Survivors, day 14

\(^1\) IC_{50}/IC_{90} (in ng/ml)

\(^2\) Approximately 10% concentration of (5.26A).

Biological evaluation of mycalamide (5.26A) in antiviral and antimicrobial assays has revealed that it expresses antiviral activity but does not exhibit antibacterial activity (Perry et al. 1988; S. Cross and P. McCarthy, unpublished observations). Mycalamide A (5.26A) exhibited in vitro antiviral activity (Table 5–5) against Herpes simplex Type 1, vesicular stomatitis, polio vaccine Type 1, coronavirus A59, and HIV; the latter assessment was carried out at NCI/NIH. In vivo activity was observed against murine coronavirus A59.

The mycalamides and onnamide have been synthesized (Hong and Kishi 1990, 1991).

5.5 COMPOUNDS IN CLINICAL TRIAL

The structures and biological activities of several marine-derived compounds that have been or are currently in clinical trials, including bryostatin 1 (5.29A), didemnin B (5.30B), manoalide (5.12), and the pseudopterosins (including A, (5.32A) and E, (5.32B)) are presented in the following sections.

5.5.1 Bryostatins

The bryostatins are a series of 15 structurally related macrocyclic lactones that are derived from the bryozoan, Bugula neritina, and have been studied for their antitumor and immunomodulatory properties for over 20 years by G.R. Pettit and his research group at the University of Arizona, scientists at NCI, and collaborators. The history of bryostatin research has been previously reviewed (Suffness et al. 1989). The bryostatins, e.g., bryostatin 1 (5.29A)
The antitumor properties of extracts from *B. neritina* were first demonstrated in 1969 using P-388 murine leukemia (Suffness et al. 1989). In one experiment, a %T/C of 168 (400 mg/kg, QD1-10) was observed. However, cytotoxicity was not observed in the KB cell line, which was used routinely at NCI in other projects to monitor fractionation of active extracts. Further, reproducible activity could not be achieved from the evaluation of extracts obtained from different collections of *B. neritina*, and research on this organism was put aside for several years.

Ten years passed before samples could be found that were sufficiently potent in in vitro assays, reproducible in in vivo antitumor assays, and in sufficient quantities to permit bioassay-guided isolation of the active components (Suffness et al. 1989). The bryostatins were also discovered in three other organisms in or on which *B. neritina* grew, i.e., the bryozoan *Amathia convoluta* (Pettit et al. 1985), the sponge *Lissodendoryx isodictyalis* (Pettit et al. 1986a), and the tunicate *Aplidium convoluta* (Pettit et al. 1986b).

In the early 1980s, bryostatins 1 and 2 (5.29B) were evaluated in a number of in vivo tumor models. Activity was demonstrated in several experimental leukemia and sarcoma models (Suffness et al. 1989); however, none of the animals were cured of their respective tumors. At the same time, similar activity was not observed against melanoma, mammary, colon, or lung cancers. This profile of biological activity was not encouraging until the results
of some of the first mechanism-of-action studies were reported beginning in 1985 (Berkow and Kraft 1985). It was also then that bryostatins 1 and 2 showed very strong specificity in NCI’s disease-specific panel against human cell lines with differences in IC$_{50}$ values between sensitive and resistant lines equal to or greater than 3 log units (Suffness et al. 1989).

The first mechanistic studies (Berkow and Kraft 1985; Kraft et al. 1986) showed that bryostatin 1 (1) binds to the phorbol ester receptor of human polymorphonuclear leukocytes and HL60 cells, which is the equivalent of the calcium, phospholipid-dependent protein kinase, i.e., protein kinase C, (2) blocked the phorbol ester-induced macrophage-like differentiation of HL-60 cells, although bryostatin 1 was unable to induce differentiation of HL-60 cells, and (3) decreased cytoplasmic protein kinase C activity. In later studies (Dale et al. 1989; Dale and Gescher 1989), the bryostatins were found to inhibit the growth of A549 lung cancer cells and to inhibit DNA replication, causing translocation of protein kinase C from the cytosol to membranes, and down regulation of protein kinase C, activity similar to that induced by phorbol esters. However, at relatively high concentrations, the bryostatins blocked their own inhibitory effect and the antireplicative action of phorbol esters. Therefore, the relationship between growth arrest and protein kinase C activities was not viewed as a straightforward one.

In addition to acting as cytotoxic and antitumor agents, and as antitumor promoters, the bryostatins modulate the immune system, presumably by affecting protein kinase C, an important mediator of signal transduction-associated cell regulation and transformation (Gescher and Dale 1989). In one study (May et al. 1987), several of the bryostatins and recombinant human granulocyte macrophage colony-stimulating factor (rHGM-CSF) were found to stimulate the formation of granulocyte-macrophage and pure granulocyte colonies in bone marrow. However, unlike rHGM-CSF, bryostatin 1 (but not bryostatin 13), was found to rapidly activate mature neutrophils, which are involved in nonspecific immune defense (Schindler 1990). This study concluded that the bryostatins may represent clinically attractive agents to complement other compounds used to treat clinical situations resulting in bone marrow failure. Bryostatin may also be useful as an adjuvant in these clinical situations. In another study (Trenn et al. 1988), the bryostatins, like the phorbol esters, were found to:

1. Lower the amount of recombinant IL-2 (rIL-2) required to generate antigen-specific, cytotoxic T-lymphocytes from in vivo primed spleen cells.
2. Cause antigen- nonspecific lysis of EL-4 cells through stimulation of antigen primed, cytotoxic T-cells when used in combination with rIL-2 and in the presence of the nonspecific T-cell activator, Con A.
3. Cause antigen- nonspecific lysis of EL-4 cells with the T-cell clone 2C.
4. Synergize with recombinant B cell stimulatory factor 1/interleukin 4 (BSF-1/IL-4), with and without rIL-2, to differentiate unprimed, resting T-cells into cytotoxic T lymphocytes.
The study concluded that the antineoplastic activities of the bryostatins themselves may be related to their immunoenhancing properties, and that clinically, they may be useful in combination with recombinant IL-2 to promote tumor rejection at IL-2 doses sufficiently low to avoid side effects. In neither study were the bryostatins found to be lymphotoxic at concentrations in which they expressed their immunomodulating effects.

Currently, through a license from G.R. Pettit/Arizona State University and under a Cooperative Research and Development Agreement (CRADA) with NCI, Bristol-Myers Squibb is pursuing large-scale isolation of the bryostatins and development (Persinos 1990d). Responding to Pettit’s encouragement in the mid-1980s, the Cancer Research Campaign in Great Britain conducted preclinical toxicology in small animals (Persinos 1989b), and clinical trials began in Manchester and Cambridge, England in February 1991 (Persinos 1991a). Bristol-Myers Squibb plans to initiate studies in dogs (Persinos 1990d), and NCI has planned more in vivo studies in animals (Persinos 1991a).

5.5.2 Didemnins

From several hundred shallow water samples of various marine organisms collected in 1978 throughout the western Caribbean, organic extracts from the tunicate *Trididemnum solidum* Van Name were found to be active in a shipboard assay against *Herpes simplex* I and cytotoxic against the viral host CV-1 cell line (monkey kidney tissue) (Rinehart et al. 1981a). In subsequent land-based assays, the tunicate extracts also exhibited in vitro activity against L1210 murine leukemia and against various RNA and DNA viruses, i.e., HSV-2, vaccinia, influenza (PR8), parainfluenza-3 (HA-1), Coxsackie (A-21), and equine rhinovirus. Three compounds were isolated initially, didemnins A through C (5.30A, 5.30B, and 5.30C), which contained both the antiviral activity and the cytotoxicity of the crude extracts (Rinehart et al. 1981b, 1981c). Structurally related compounds didemnins D and E were subsequently isolated and their structures elucidated (Rinehart et al. 1988). A total of 12 didemnins have been detected (Munro et al. 1987). The didemnins are all cyclic depsipeptides with five or more amino acid units in common. Didemnin D, the largest compound in the series whose structure has been published, contains 12 amino acid units, including standard amino acids (L-Glu, L-Thr, L-Leu, L-Pro), methylated amino acids (D-MeLeu, N,O-dimethyl-L-Tyr), and unusual groups (L-Lac-L-Pro, (3S,4R,5S)-isostatine, and (2S,4S)-hydroxyisovalerylpropionic acid). The isostatine unit was originally thought to be (3S,4R)-statine (Rinehart et al. 1988), and the hydroxyisovalerylpropionic acid was tentatively originally assigned as the 2R,4R-diastereomer and subsequently revised (Ewing et al. 1986).

In addition to antiviral activity and cytotoxicity, the didemnins exhibit antitumor and immunomodulatory activity; the antiviral activity was pursued initially. In the original reports (Rinehart et al. 1981b, 1981c), didemnins A
and B exhibited good dose-response relationships against the viruses in which the crude extracts were active (vide supra). Subsequent in vitro studies revealed activity against a broad range of RNA viruses, including Rift Valley fever, Venezuelan equine encephalomyelitis virus, yellow fever, sandfly fever, and a Pichinde virus (Canonico et al. 1982). In vivo activity was demonstrated by didemnin B against lethal challenges of HSV-2 (DNA virus) in the mouse vaginal Herpes model (Canonico et al. 1982; Weed and Stringfellow 1983), and against Rift Valley fever (RNA virus) (Rinehart et al. 1988). Unfortunately, didemnin B was only effective in vivo against Herpes if topical applications were started prior to infection, and was not effective if applied after infection or given intraperitoneally. In general, the didemnins were found to be too toxic and not sufficiently selective for antiviral therapy (Rinehart et al. 1988; Munro et al. 1987).

The immunomodulatory properties of the didemnins were studied initially by researchers at the University of Arizona (Montgomery and Zukoski 1985; Montgomery et al. 1987a, 1987b; Russell et al. 1987). In the first published study by this group (Montgomery and Zukoski 1985), didemnin B was found to inhibit the alloantigen response in the MLR, inhibit Con A-induced blastogenesis of T lymphocytes, and inhibit bacterial lipopolysaccharide (LPS)-induced blastogenesis of B lymphocytes at nanogram to picogram/milliliter concentrations, which were nontoxic to unstimulated lymphocytes. Didemnin B also inhibited protein synthesis in normal resting splenic mononuclear cells, but at concentrations much greater than those affecting Con A- and LPS-induced mitogenesis, and did not affect RNA synthesis at microgram/milliliter concentrations. Didemnin B also inhibited splenomegaly in the in vivo Simonsen GVHR at 0.05 to 0.3 mg/kg/day; however, concentrations of didem-
nin B of ≥0.2 mg/kg/day caused mortality, primarily through adverse effects on hepatic function.

Follow-up in vivo studies of the inhibition of LPS-induced mitogenesis were conducted (Montgomery et al. 1987a, 1987b). Observed changes in bone marrow function due to didemnin B were thought to be related to increased production and release of lymphocytes and neutrophils from bone marrow. From a study by a different research group to investigate the mechanism of inhibition of T-lymphocyte proliferation (LeGrue et al. 1988), didemnin B was found to inhibit lymphocyte proliferation through a cytostatic affect; its mechanism of inhibition appears to be distinct from that of cyclosporine, which affects lymphokine production.

Other in vivo studies have yielded mixed results. In one study, didemnin B was found to significantly prolong survival of rat heart allografts with doses as low as 0.005 mg/kg; however, long-term survival of rat heart allografts could not be achieved, even at near-lethal dosages, which contrasts with the effects of cyclosporine (Stevens et al. 1989). In another study (Yuh et al. 1989), didemnin B was evaluated in mouse and rat heterotopic cardiac transplantation models and the mouse popliteal lymph node hyperplasia assay and found to possess a low therapeutic index, i.e., doses of didemnin B that prolonged allograft survival or inhibited lymphocyte proliferation were quite toxic, evidenced by significant (reversible) body weight loss. Toxicity due to didemnin B appeared to be cumulative, which was presumed to be related to the lipophilicity and, therefore, accumulation of the compound in certain body tissues. The latter study concluded that the in vitro potency of didemnin B was (unfortunately) not sufficient to predict its efficacy as an immunosuppressant in vivo and that a suitable mechanism of action, i.e., as shown by the clinically used agent, cyclosporine, is highly important.

The most advanced area of biological evaluation of the didemnins is as an antitumor agent. The cytotoxic and antitumor properties and results from mechanism-of-action studies of the didemnins have been previously reviewed (Chun et al. 1986; Munro et al. 1987; Rinehart et al. 1988). Didemnins A and B are potent cytotoxins against L1210 with ED$_{50}$ values of 30 and 2 ng/mL, respectively. In vivo activity was established for didemnin B in (i.p.) P-388 murine leukemia (%T/C = 199, 1 mg/kg, i.p., Q1,5,9, (i.p.) B16 murine melanoma (%T/C = 160, 1 mg/kg, i.p., Q1,5,9; %T/C = 172, 0.3 mg/kg, i.p., QD1-9), and (i.p.) M5076 murine sarcoma (%T/C = 209, Q1,5,9,13). Didemnin B also exhibited in vivo activity in (i.p.) Yoshida ascites tumors in rats (%T/C = 369, 0.06 mg/kg, i.p., Q1, 11 deaths/20 rats total) (Fimiani 1987). In vivo efficacy was not observed in experiments with subcutaneous (s.c.) CD8F murine mammary tumor, (s.c.) colon 38 tumor, (i.p.) L1210 murine leukemia, intravenous (i.v.) Lewis lung carcinoma, CX-1 human colon tumor (xenograft), LX-1 human lung tumor (xenograft), and MX-1 human mammary tumor (xenograft). Didemnin A has not been tested extensively in in vivo tumor models, and in experiments in which it has been evaluated, it has proven not to be as efficacious as didemnin B.
Antitumor activity against B16 murine melanoma was not found for didemnin B with subcutaneous, intravenous, or oral administration; none of the in vivo experiments yielded long-term survivors, and the dosages in the P-388 and B16 experiments that provided the greatest treatment values, i.e., 1 mg/kg, also caused the greatest amount of weight loss (Chun et al. 1986; Rinehart et al. 1988).

Didemnin B has also been evaluated in the human tumor stem cell or human tumor colony forming assays using fresh human tumor surgical or biopsy specimens (results also reviewed in Munro et al. 1987 and Chun et al. 1986). Activity at nanogram/milliliter to microgram/milliliter concentrations were observed against carcinomas of the breast, ovary, and kidney, mesothelioma and sarcoma, ovarian cancer, hairy cell leukemia, breast cancer, and oligodendroglioma.

Didemnin B appears to exert its antiproliferative effects in various cell lines primarily through inhibition of protein synthesis and to a lesser extent through inhibition of DNA synthesis (reviewed in Chun et al. 1986). Inhibition of protein synthesis is not related to amino acid uptake and didemnin B does not bind to DNA. The compound effects exponentially growing B16 cells to a much greater extent than plateau-phase cells. Low doses of didemnin B stop B16 cell progression at the G1/S boundary while cell progression from S to G2 and M to G1 is relatively unaffected, except at high doses when complete inhibition of cell progression or growth occurs.

Didemnin B has completed Phase I clinical trials as an anticancer agent (Dorr et al. 1988). The dose-limiting toxicity was nausea and vomiting, which could be ameliorated somewhat by pretreatment with an aggressive antiemetic regimen; no objective antitumor response was observed.

Broad Phase II clinical trials for didemnin B as an antitumor agent are ongoing (Persinos 1991c).

5.5.3 Manoalide
The sesterterpenoid manoalide (5.12) and structurally related compounds were obtained from the marine sponge, *Luffariella variabilis* (de Silva and Scheuer 1980, 1981); the compound was named in recognition of the location in which the chemistry work was completed (Manoa Valley, Hawaii). Although manoalide and the crude methylene chloride extract from which it was derived demonstrated in vitro antibiotic activity against *Streptomyces pyogenes* and *Staphylococcus aureus* (de Silva and Scheuer 1980), it was the analgesic and anti-inflammatory activity of manoalide (Faulkner 1992; Jacobs et al. 1985) and a related series of compounds (Albizati et al. 1987; Kernan et al. 1987) that elicited substantial scientific and pharmaceutical interest. The first total synthesis of manoalide and the related compound, seco-manoalide, was accomplished soon after the structure was published (Katsumura et al. 1985) and thus ameliorated any anticipated difficulties regarding supply for
in-depth biological evaluation and facilitated the possibility of preparing more efficacious analogs.

The analgesic activity of manoalide was demonstrated in the phenylquinone-induced writhing assay in mice, with an $ED_{50} = 0.36$ mg/kg, i.p. (Jacobs et al. 1985). Manoalide was also shown to be active in antagonizing the inflammatory response of mouse epidermis induced by PMA, with potency greater ($ED_{50} = 100 \, \mu g$) than that of the standard nonsteroidal compound, indomethacin ($ED_{50} = 250 \, \mu g$) but less than that of hydrocortisone ($ED_{50} = 20 \, \mu g$, Burley et al. 1982). Manoalide, like hydrocortisone, did not prevent the inflammation response normally induced by arachidonic acid, and it was postulated that the compound might inhibit enzyme reactions that were located upstream from arachidonic acid release and prostaglandin synthesis. This hypothesis was supported, in part, by in vivo time course studies showing that manoalide was most effective when applied within 5 minutes of PMA application, a very early time point considering the normal 3-hour period it required for the inflammatory reaction to manifest itself.

Speculation at that time on the mechanism of action of both indomethacin, which prevents prostaglandin production by inhibition of the cyclooxygenase pathway, and hydrocortisone, which prevents prostaglandin production by inhibiting arachidonic acid release, led to the hypothesis that manoalide might serve to inactivate or inhibit various actions of the enzyme PLA$_2$. In one study (de Freitas et al. 1984), manoalide ($2 \times 10^{-5}$ M) was shown to prevent the neurotoxic action of $\beta$-bungarotoxin on rat phrenic nerve-diaphragm preparations when preincubated with the toxin for as little as 25 minutes. In the same study, manoalide inhibited the hydrolysis of phosphatidylcholine by bee venom-derived PLA$_2$, when preincubated with the enzyme for 1 hour. Subsequent studies demonstrated the irreversibility of the reaction and its pH dependence (Glaser and Jacobs 1984, 1986). Manoalide has also been shown to inhibit the activity of PLA$_2$ from other sources, including cobra venom (Lombardo and Dennis 1985), while manoalide blocked the hydrolysis of both phosphatidylcholine and phosphatidylethanolamine by bee venom PLA$_2$.

Manoalide has also been reported to be 300- to 100-fold less active against PLA$_2$ obtained from mammalian microsomal preparations (guinea pig lung and uterus rat basophilic leukemia cells and smooth muscle) than against PLA$_2$ obtained from cobra venom (Bennett et al. 1987). These results suggest that PLA$_2$ alone is not the sole principal intracellular target for manoalide. Further, manoalide was found to be capable of inhibiting phosphoinositide-specific phospholipase C (PLC), within an $IC_{50}$ value range of from 3 to 6 $\mu$M; this inactivation was calcium and pH dependent and irreversible.

While the purported mechanism of action of manoalide's anti-inflammatory activity appeared to involve direct binding of the molecule to PLA$_2$, manoalide was observed to form a chromophore when mixed with bee venom PLA$_2$ (Glaser and Jacobs 1986). A similar reaction occurred when manoalide was incubated with monomeric lysine, cysteine, and tryptophan, but not with their respective N-alpha-amino-blocked analogs. Through a series of straight-
forward but elegant experiments, a correlation between chromophore production and the specific amino acid residues modified upon binding of manaoalide to \( \text{PLA}_2 \) and corresponding enzymatic activity guided the team to this discovery. The results of these and subsequent experiments (Glaser et al. 1988) indicated that of the 11 lysine residues available for modification by manaoalide, only three were actually modified upon binding by manaoalide to bee venom \( \text{PLA}_2 \). This contrasted with the modification of four of the six lysines present in cobra venom \( \text{PLA}_2 \) (Lombardo and Dennis 1985). Subsequent investigations (Glaser et al. 1989) have revealed a number of structure activity relationships associated with the manaoalide molecule and related analogs that may prove useful in determining the exact nature of the binding of manaoalide to \( \text{PLA}_2 \). These include the \( \gamma \)-hydroxybutenolide ring system (selectivity for various sources of \( \text{PLA}_2 \)), the C-24 aldehyde (the irreversibility of \( \text{PLA}_2 \) binding), and the hydrophobic alkyl regions of the molecule (cooperative binding with the ring system and \( \text{PLA}_2 \) molecule).

Manaoalide’s mechanism of the inhibition of phospholipid hydrolysis led to a number of investigations into its utility as a probe to study the role and importance of phospholipases and the role of \( \text{Ca}^{2+} \) in signal transduction-mediated events during cellular growth and replication. A key component of many inflammatory mediators and growth factors is their ability to bind to cellular receptors that, in turn, stimulate phosphoinositide turnover and \( \text{Ca}^{2+} \) mobilization as part of the signal transduction pathway. The major role of PLC in this process includes the activation of phosphatidylinositol-4,5-bisphosphate (\( \text{PIP}_2 \)), which gives rise to inositol 1,4,5-triphosphate (\( \text{IP}_3 \)) and 1,2 diacylglycerol (DAG) (Berridge 1983). The \( \text{IP}_3 \) serves as a second messenger and binds to a receptor on the rough endoplasmic reticulum, which causes the release of \( \text{Ca}^{2+} \) from intracellular stores. DAG, on the other hand, is the physiological receptor for protein kinase C, an important second messenger enzyme involved in the regulation of cellular proliferation. While manaoalide’s primary pharmacological action involves inhibition of \( \text{PLA}_2 \) activity, as previously noted, manaoalide’s less potent, but measureable inhibition of PLC activity prompted further investigations into its role as an antagonist of calcium-mediated, signal transduction-mediated control of cellular growth and regulation.

Manaoalide was found to be a potent inhibitor of \( \text{Ca}^{2+} \) mobilization in several cell lines (Wheeler et al. 1987). Epidermal growth factor (EGF) stimulates a rise in intracellular \( \text{Ca}^{2+} \) in A431 human epidermoid carcinoma cells. Manaoalide blocked both the EGF-mediated entry and release of \( \text{Ca}^{2+} \) from intracellular stores in a time-dependent manner with an IC\(_{50}\) of 0.4 \( \mu \text{M} \). However, the production of inositol monophosphate (phosphoinositide metabolism) did not coincide with the EGF-mediated response. Manaoalide also blocked the thyrotropin-releasing hormone (TRH)-dependent release of \( \text{Ca}^{2+} \) from intracellular stores in \( \text{GH}_3 \) rat pituitary cells (0.5 \( \mu \text{M} \) for 20 minutes or 3.0 \( \mu \text{M} \) for 5 minutes), without concomitant inhibition of the formation of inositol phosphates from \( \text{PIP}_2 \). In addition, manaoalide also inhibited the K\(^+\)
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depolarization-activated Ca\(^{2+}\) channel in these cells and the activation of the channel by BAY K8644, with an IC\(_{50}\) = 1 μM. Manoalide also inhibited Ca\(^{2+}\) uptake of murine splenocytes stimulated with concanavalin A in a time- and temperature-dependent manner with an IC\(_{50}\) = 0.07 μM, thus indicating that at least in the cells examined in the study, manoalide’s inhibitory action appeared to involve blockage of the calcium channel.

The role of manoalide in the regulation of various eicosanoids and their precursors and the role they play in the inflammatory process began to be established several years ago (Mayer and Jacobs 1988; Mayer et al. 1988). Manoalide inhibited the production of prostaglandin E\(_2\) (PGE\(_2\)) in a dose-dependent fashion by PMA-zymosan stimulated murine peritoneal macrophages. The release of leukotriene C\(_4\) (LTC\(_4\)) in murine peritoneal macrophages stimulated with the calcium ionophore A23187 was similarly inhibited. Curiously, LTC\(_4\) production was enhanced by manoalide when the same cells were stimulated with zymosan. Both PGE\(_2\) and LTC\(_4\) production were reduced in resident (unstimulated) mouse peritoneal macrophages, indicating a direct inhibitory effect of manoalide on the cyclooxygenase pathway. Additionally, the release of \(^3\)H-arachidonic acid was shown to be partially reduced (37% inhibition) in the presence of 0.05 μM of manoalide. These results pointed to the possibility that manoalide’s mechanism of action appeared to include all of the following:

1. Inhibition of PLC and PLA\(_2\);
2. The resulting inhibition of arachidonic release;
3. Inhibition of PGE\(_2\) production; and
4. Blockage of Ca\(^{2+}\) mobilization.

Subsequent studies (Lister et al. 1989) confirmed and extended the findings of Mayer using the murine macrophage cell line P388D\(_1\). Lister found that manoalide, unlike other PLA\(_2\) inhibitors, was equally effective in inhibition of both intracellular and extracellular (isolated) PLA\(_2\).

While manoalide continues to be a useful tool in helping to elucidate the complex interplay of eicosanoid regulation and membrane phospholipid-signal transduction mediated events, the in vivo anti-inflammatory activity of the compound and its related analogs suggest the potential clinical usefulness of the compound in disorders that manifest themselves through altered production of inflammatory eicosanoid products (i.e., prostaglandins). In vitro studies (Jacobson et al. 1990) in which manoalide was demonstrated to inhibit PLA\(_2\) from human synovial fluid, the site of pathological damage in rheumatoid arthritis, and the demonstration of manoalide’s ability to inhibit the release of elastase from human neutrophils stimulated by fMLP (fMet-Leu-Phe) further underscore the potential of the compound as a potential next generation anti-inflammatory agent. In the past 5 years, a tremendous number of synthetic analogs of manoalide have been prepared (Lee 1990a, 1990b; Maullem et al. 1989), including manoalogue (5.31) (Reynolds et al. 1988).
Currently, manoalide and selected analogs are being evaluated in clinical trials as topical anti-inflammatory agents (R. Jacobs, personal communication).

### 5.5.4 Pseudopterosins

The pseudopterosins are a series of 12 tricyclic diterpene glycosides (A–L) that were isolated from the Caribbean gorgonian, *Pseudopterogorgia elisabethae* (Look et al. 1986a, 1986b, Roussis et al. 1990), based on the biological activity of the crude extract. Pseudopterosin A (5.32A) contains a 3-O-β-D-xylopyranose, which is attached to the diterpene group at C-9; pseudopterosins B through D are structurally identical, except that the xylopyranose is monoacetylated at the various hydroxyl moieties. Pseudopterosins G through J are diastereomers of A through D with a β-CH₃ at C-7 and contain regioisomeric acetates of an α-L-fucose group attached at C-9; pseudopterosins K and L also contain an α-L-fucose group, which is attached at C-9, and in the case of L, is monoacetylated. However, they are diastereomers of A.
through D with an α-isopropenyl group at C-1, a β-CH₃ at C-3, and a β-CH₃ at C-7; pseudopterosins E (5.32B) and F contain identical diterpenoid groups as found in A through D; however, E contains an α-D-arabinose, which is attached to C-10 and F contains an α-L-fucose at C-10. The biological activities first reported about the purified compounds described both antimicrobial activity and cytotoxicity directed toward fertilized eggs from the California sea urchin (Ettouati and Jacobs 1987; Jacobs et al. 1981; Jacobs and Wilson 1986). However, potent anti-inflammatory and analgesic activities of pseudopterosin A (5.32A) (Look et al. 1986a) further drove the biological and chemical characterization of these compounds.

Initial studies (Look et al. 1986a) indicated that pseudopterosin A (5.32A) was significantly more potent in blocking the phorbol myristate acetate, topically induced inflammatory response of mouse skin (K = 8.9 × 10⁻⁴ M) compared with the standard anti-inflammatory drug indomethacin (40 mM). Concentrations of pseudopterosin A greater than 100 μM did not inactivate the hydrolysis of phosphatidyl choline by phospholipase A₂ (PLA₂) from bee venom or liver microsomal preparations; however, pancreatic PLA₂ activity was shown to be inhibited, with IC₅₀ values ranging from 3.0 to >80 μM.

Pseudopterosin A (5.32A) was also found to be more potent than indomethacin as an analgesic for blocking the stretch-flex response of phenylquinone injected mice (ED₅₀ obtained at 3.1 mg/kg of pseudopterosin A versus 10 mg/kg for indomethacin). Pseudopterosin A did not mimic morphine in electrically driven preparations, nor antagonized histamine or bradykinin at concentrations up to 30 μM, and did not prevent phorbol dibutyrate inhibition of bradykinin-induced contractions of the guinea pig ileum.

While pseudopterosins A through D expressed some in vivo toxicity (acute toxicity in the range of 50 mg/kg in mice), pseudopterosin E (5.32B) demonstrated very low acute toxicity in mice (LD₅₀ > 300 mg/kg) and an anti-inflammatory potency equivalent to that of pseudopterosin A (Roussis et al. 1990). In addition, the preliminary biological activities of pseudopterosin E suggest a unique mechanism of action via the inhibition of leukotriene synthesis. While the precise mechanism of action of the compound is unknown, the molecule seems to serve as a potential antagonist of the lipoxygenases or other enzymes involved in the arachidonic cascade.

The superior in vivo and in vitro anti-inflammatory activities of pseudopterosin E prompted several investigations into the possible derivatization and/or synthesis of the molecule. An attempt was made to obtain the compound by the interconversion of pseudopterosin A to E (Roussis et al. 1990), however, the inefficiency of glycosidation reactions lead to poor yields of the compound. The first total enantiospecific synthesis of pseudopterosin E was reported through the direct attachment of an L-fucose unit to the aglycone via a novel method (Corey and Carpino 1989). A method for the synthesis of pseudopterosin A was similarly described. Other investigations revealed alternate pathways for obtaining both the glycosylated form and the aglycone of pseudopterosin E (Broka et al. 1988; Ganguly et al. 1990). The importance
of the role of glycosylation of the molecule is underlined by the fact that the aglycone portion, alone, is not active in vivo or in vitro.

With the synthesis of these compounds well in hand, work continues on the synthesis of various analogs of these compounds and the determination of the precise mechanism of action of their anti-inflammatory activities (R. Jacobs, personal communication). Present evidence indicates that one of the primary mechanisms of the anti-inflammatory effects of the pseudopterosins lies in its ability to selectively block degranulation and leukotriene production in human polymorphonuclear neutrophils (R. Jacobs, personal communication). The model compound, pseudopterosin E, has no effect on eicosanoid biosynthesis in cultured mouse peritoneal macrophages (R. Jacobs, personal communication).

Currently, the pseudopterosins are undergoing clinical evaluation for topical use (R. Jacobs, personal communication).

5.6 CLINICAL AGENTS RESEMBLING MARINE-DERIVED COMPOUNDS

5.6.1 Adenine Arabinoside (Ara-A) and Cytosine Arabinoside (Ara-C)

From studies of Caribbean sponge sterols by W. Bergmann and co-workers in the early 1950s (Bergmann and Burke 1956; Bergmann and Feeney 1950, 1951; Cohen 1966), the nucleosides spongouridine (5.33A) and spongothymidine (5.33B) were discovered serendipitously and found to possess antiviral activity. These discoveries aided in the development of antitumor agents and a generation of nucleoside antivirals. Through sporadic synthetic analog efforts to improve the activities of the compounds, cytosine arabinoside (Ara-C) (5.33C) was introduced nearly 20 years later as a clinically useful antitumor agent (Bodey et al. 1969), and nearly 30 years later, adenine arabinoside (Ara-A) (5.33D) was approved for use as an antiviral agent (Buchanan and Hess 1980). Cytosine arabinoside is produced synthetically, currently distributed as Cytosar-U® and indicated in acute nonlymphocytic leukemia, chronic myelocytic leukemia, and meningeal leukemia (Physicians Desk Reference 1991). Adenine arabinoside is obtained from fermentation cultures of Streptomyces antibioticus and currently distributed as Vira-A® (Physicians Desk Reference 1991). Through intravenous administration, Vira-A is indicated for the treatment of Herpes simplex virus encephalitis, Herpes simplex infections in newborns, and Herpes zoster virus due to reactivated varicela-zoster virus infections in immunosuppressed patients. As an ointment, Vira-A® is used for the topical treatment of epithelial keratitis caused by Herpes simplex. Subsequent second- and third-generation nucleosides, i.e., acyclovir, AZT and dideoxycytidine, are highly useful antiviral agents and the objects of further medicinal chemistry studies to find even more efficacious and selective compounds.
In contrast to biologically active compounds from terrestrial organisms, on which a considerable amount of work has been done and success achieved, the knowledge base of biological information about compounds from marine organisms is very limited. However, for the few compounds that have been studied in detail, the success rate is high and the potential is considerable. Over the past three decades, over 4000 novel compounds have been described from marine organisms (reviews by Faulkner 1984a, 1984b, 1986, 1987, 1988, 1990, 1991), some of which possess striking biological activities, and have been described in this chapter. In addition, recent reports on the results of marine extract screening strongly suggest that the marine environment holds promise for a continued high abundance of biologically active compounds (Munro et al. 1987). Marine natural products, due to their unrivaled degree of structural novelty, have served to inspire many of the great synthetic achievements in organic chemistry. Thus, with the great structural novelty and high degree of biological activity associated with this group of substances,
why is it that, in the past 5 to 10 years, there have been no new marine-derived compounds that began as NCEs (new chemical entities) in the United States on the market?

The current role of marine natural products in the therapeutic regime must be considered in light of drug discovery and development as a whole. Given the time and capital investment required to discover, develop, and market new drugs (Niel 1988), it is a natural consequence that therapeutic agents on today's market are, for the most part, the result of research and development efforts of 5, 10, or more years hence, and, unfortunately, much of the published biological screening effort during this time period on marine-derived compounds has been mostly classical. Indeed, marine-derived compounds of current clinical interest (see Section 5.5) are the products of marine drug discovery programs smaller in scope and number than those that exist today, and most of the current industrial marine drug discovery efforts were initiated just within the last 5 years. The field is only now beginning to evolve to a state of relative maturity and the total chronology of recent marine drug discovery efforts has yet to run its course.

Consideration of the numerical parameters of drug discovery also reconciles the current minor role played by marine agents in chemotherapy. In 1970, over 703,000 substances were pharmacologically tested by the pharmaceutical industry (Reuben and Wittcoff 1989). The overall composition of this total is somewhat unclear since it is likely comprised of numerous sources such as extracts of fermentation products, natural substances, pure synthetics, etc., and statistical comparisons must be interpreted with caution. It is clear, however, that even today's heightened level of marine-derived compound and extract screening (a few thousand per year perhaps) represents only a small portion of this amount. Consideration of the scope of therapeutic areas screened shows a parallel situation. During the 1970s and early 1980s, marine screening efforts were focused, with some exceptions, in the areas of anti-tumor, cytotoxics, antiviral, and antimicrobials. These areas represent only a portion of the full range of human disease areas. Today, in addition to these, investigations of marine products in immunomodulation, inflammation, cardiovascular, central nervous system, hypertension, to name only a few, are now well underway. These efforts set the stage for marine compounds to play an increasingly important role in disease chemotherapy in the years to come.

Having seen that overall research efforts in marine drug discovery are on the rise, we briefly consider here several possible scenarios in which a marine natural product may lead to a therapeutic agent.

5.7.1 A Marine-Derived Compound that Becomes a Successful Drug

There are diseases such as AIDS, certain forms of cancer, Alzheimer’s disease, and others where no truly effective form of chemotherapy exists and where
an unoptimized natural product may comprise a suitable treatment. In these cases, the acute need for effective agents would provide the driving force to accelerate clinical investigations of relatively complex, naturally occurring agents.

In the usual commercial scenario, natural products that are biologically active and structurally complex are found in microorganisms and produced in large quantities by fermentation, or they are found in plants and produced in large quantities by cultivation. If the incentives are great enough, total synthesis of a complex natural product is undertaken; for example, the immune suppressant, FK506 (Jones et al. 1990; Nakatsuka et al. 1990). In contrast, pure didemnin B and bryostatin 1 were obtained for clinical trials from massive field collections of the source organisms Trididemnum solidum (Rinehart et al. 1988) and Bugula neritina (Schaufelberger et al. 1991), respectively. Didemnin B and bryostatin 1 serve well to illustrate a central problem of marine drug development, that of compound supply. While the biological richness of the marine environment is well appreciated, the prospects are usually not good for producing large amounts (kilograms) of marine compounds using field collection of organisms. Only the most abundant marine organisms can be collected in these amounts. An additional complication is the well-established variation in chemistry within a species as a function of geographical location. Thus, only in select cases with highly abundant organisms (i.e., some sponges, bryozoans, gorgonians, or algae) are extremely large-scale collections possible, and even then, environmental consequences can be severe. Therefore, while in some cases it might be possible to produce amounts of marine compound sufficient for preliminary clinical trial (gram quantities) from collection of the source organism, this approach cannot produce quantities needed for extended trials and the market (tens of kilograms).

Several, exciting new technologies hold promise for solving the supply problem, i.e., sponge cell culture and aquaculture, but it is presumed that current technologies of chemical synthesis and microbial fermentation will provide the first solutions. Fermentation is a mainstay in the pharmaceutical industry for compound production, in many cases providing supplies of structurally complex antibiotics, antitumor antibiotics, and immune suppressants, which are economically inaccessible by chemical synthesis. Fermentation of marine microorganisms may provide additional benefits outside drug development. An example of this is the culture of thermophilic bacteria isolated from marine geothermal sites (Borman 1991), whose thermally robust enzymes may have potential utility as industrial catalysts for the production of fuels, pharmaceuticals, or specialty chemicals. Indeed, the origin of several bioactive marine natural products isolated from sponges (Stierle et al. 1988; Elyakov et al. 1991) and other macroorganisms (Tamplin 1990) have been traced to microbial symbionts, and reports of success in the culture of these microorganisms are becoming more frequent.

The central role of chemical synthesis in drug development certainly requires no illustration. Chemical synthesis or modification of natural com-

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pounds yields the majority of chemotherapeutic agents in the U.S. market, especially in certain disease areas. Due to the inherent supply problem described previously, even in cases where the original, parent compound might be a suitable drug candidate, chemical synthesis is still likely to become the means by which suitable quantities are produced for advanced clinical investigation and marketing. The natural outgrowth of such a synthetic effort is the investigation of analogs as part of a medicinal chemistry effort. This brings forth another scenario.

5.7.2 A Bioactive Marine Natural Product as a Structural Lead

Many marine natural products have striking molecular complexity to match their biological activity. Certainly, molecules such as palytoxin (Armstrong et al. 1989a, 1989b), mycalamide (Hong and Kishi 1990), onnamide (Hong and Kishi 1991), and okadaic acid (Isobe et al. 1987) represent tremendous challenges to the synthetic organic chemist. The fact that many complex marine-derived molecules have now been synthesized is a testimony to the great advances made in the science and art of synthetic chemistry; however, even if compounds of this type did possess medicinal value competitive with current agents, their structural complexity greatly lowers the likelihood of an economically viable synthesis.

It is more likely that a bioactive, complex marine metabolite will serve as a lead structure for a medicinal chemistry effort. Programs of this type are designed with several goals in mind, not the least of which is the discovery of structural analogs exhibiting improved therapeutic attributes and simpler (i.e., more economical) chemical structures, as well as trying to secure sufficiently broad and appropriate patent coverage. An illustrative case of such a scenario is available in the case of manoalide (see Section 5.5.3), a compound isolated from the marine sponge *Luffariella variabilis* (de Silva and Scheuer 1980). Manoalide was found to be a specific inhibitor of phospholipase A\textsubscript{2} and C and is currently under development as an anti-inflammatory agent (see Section 5.5.3). The synthesis and evaluation of an extensive series of analogs was undertaken by several pharmaceutical companies and some of this work has been reported in the patent literature as well as the open literature (Garst et al. 1986; Katsumura et al. 1985; Lee 1990a, 1990b; Maullem et al. 1989). Consequently, manoalide and selected synthetic analogs have been evaluated as topical anti-inflammatory agents in clinical trials (R. Jacobs, personal communication).

Because of the enormous investment of money (>U.S. $200 million) and time (10 years) involved in the development of a new therapeutic agent that performs well, the desire to develop a better analog becomes very important. Therefore, considerable effort is expended to prepare second- and third-generation compounds that are even more active and less toxic. Similar to the situation with Ara-A and Ara-C, this scenario will certainly also apply to
newly discovered marine-derived compounds or analogs that are developed as therapeutic agents.

5.8 CONCLUSIONS

By highlighting the sources, collection methods and strategies, structures, and respective biological activities of numerous marine-derived compounds, we believe we have shown that the marine environment is a rich and viable source of structurally unusual and biologically active compounds with therapeutic potential. Further, we believe that we have touched on many of the important issues related to marine-derived therapeutic agents, including the discovery approaches, supply/synthesis versus collection or fermentation, structure-activity optimization studies, and appropriate collaboration between academic researchers and industry and between research and development groups.

Finally, we believe that an important key to realizing the therapeutical potential of marine natural products is patience; the discovery by an academic, government, or industrial research group of a marine-derived compound with a satisfactory biological profile to become a lead compound may occur tomorrow or may not occur for years, and when it is discovered, the optimized compound may take another 10 to 15 years to reach the market. The wait will be worthwhile, both scientifically and commercially, because of the numerous, inevitable spin-offs: the continued discovery and development of biochemical probes or tools, a better understanding of the biology of particular disease states based on the unique biological activities of marine-derived compounds, a better understanding of the marine organisms that produce these compounds and of their respective environments, and advances in spectroscopy and synthesis as a consequence of conducting research on these often complex and structurally unusual molecules.

REFERENCES

Abraham, E.P. (1962) Pharmacol. Rev. 14, 473–500.
Abraham, E.P., and Newton, G.G.F. (1967) in Antibiotics 2, Biosynthesis (Gottleib, D., and Shaw, P.D., eds.), pp. 1–16, Springer-Verlag, New York.
Adamczeski, M., Quinoa, E., and Crews, P. (1988) J. Am. Chem. Soc. 110, 1598–1602.
Aimi, N., Odaka, H., Sakai, S.-I., et al. (1990) J. Nat. Prod. 53, 1593–1596.
Alberts, A.W., Chen, J., Kuron, G., et al. (1980) Proc. Natl. Acad. Sci. USA 77, 3957–3961.
Albizati, K.F., Holman, T., Faulkner, D.J., Glaser, K.B., and Jacobs, R.S. (1987) Experientia (Basel) 43, 949–950.
Armstrong, R.W., Beau, J.-M., Cheon, S.H., et al. (1989a) J. Am. Chem. Soc. 111, 7525–7530.
Armstrong, R.W., Beau, J.-M., Cheon, S.H., et al. (1989b) J. Am. Chem. Soc. 111, 7530–7533.
Armstrong, J.E., Janda, K.E., Willoughby, R., et al. (1991) in Second International Marine Biotechnology Conference, Baltimore, MD, Abstract P10, Society for Industrial Microbiology, Annandale, VA. p. 80.
Baertschi, S.W., Brash, A.R., and Harris, T.M. (1989) J. Am. Chem. Soc. 111, 5003–5005.
Baker, B.J., Okuda, R.K., Yu, P.T., and Scheuer, P.J. (1985) J. Am. Chem. Soc. 107, 2976–2977.
Bakus, G.J., Targett, N.M., and Schulte, B. (1986) J. Chem. Ecol. 12, 951–987.
Barnes, R.D. (1980) Invertebrate Zoology, 4th ed., Saunders College/Holt, Rinehart and Winston, Philadelphia.
Bennet, C.F., Mong, S., Clark, M.A., Kruse, L.I., and Crooke, S.T. (1987) Biochem. Pharmacol. 36, 733–740.
Bergmann, W., and Burke, D.C. (1956) J. Org. Chem. 21, 226–228.
Bergmann, W., and Feeney, R.J. (1950) J. Am. Chem. Soc. 72, 2809–2810.
Bergmann, W., and Feeney, R.J. (1951) J. Org. Chem. 16, 981–987.
Berkow, R., and Fletcher, A.J. (1987) Merck Manual, 15th ed., Merck & Co., Rahway, NJ.
Berkow, R.L., and Kraft, A.S. (1985) Biochem. Biophys. Res. Commun. 131, 1109–1116.
Bergquist, P.G. (1978) Sponges, pp. 197–198, University of California Press, Los Angeles.
Bergquist, P.R., and Wells, R.J. (1983) in Marine Natural Products, vol. V (Scheuer, P.J., ed.), pp. 1–50, Academic Press, New York.
Berridge, M. (1983) Biochem. J. 212, 849–858.
Bodey, G.P., Freirich, E.J., Monto, R.W., and Hewlett, J.S. (1969) Cancer Chemical. 53, 59–66.
Borman, S. (1991) Chem. Eng. News 69, 31–34.
Boyd, M.R., Schoemaker, R.H., Cragg, G.M., and Suffness, M. (1988) in Pharmaceuticals and the Sea (Jefford, C.W., Rinehart, K.L., and Shield, L.S., eds.), pp. 27–44, Technomic Publishing Company, Lancaster, PA.
Broka, C.A., Chan, S., and Peterson (1988) J. Org. Chem. 53, 1584–1586.
Buchanan, R.A., and Hess, F. (1980) Science 127, 143–171.
Bundy, G.L., Daniels, E.G., Lincoln, F.H., and Pike, J.E. (1972) J. Am. Chem. Soc. 94, 2124.
Burger, A. (1986) Drugs and People, University Press of Virginia, Charlottesville.
Burley, E.S., Smith, B., Cutter, G., Ahlen, J.K., and Jacobs, R.S. (1982) Pharmacist 24, 117.
Burkholder, P.R., Pfister, R.M., and Leitz, F.H. (1966) Appl. Microbiol. 14, 649–653.
Burres, N.S., and Clement, J.J. (1989) Cancer Res. 49, 2935–2940.
Burres, N.S., Sazesh, S., Gunawardana, G.P., and Clement, J.J. (1989) Cancer Res. 49, 5267–5274.
Canonico, P.G., Pannier, W.L., Huggins, J.W., and Rinehart, K.L. (1982) Antimicrob. Agents Chemother. 22, 696–697.
Caprile, K.A. (1988) J. Vet. Pharmacol. Therap. 11, 1–32.
Cardellina, J.H., Marner, F.-J., and Moore, R.E. (1979) Science 204, 193–195.
Carmely, S., Kashman, Y., Loya, Y., and Benayahu, Y. (1980) Tetrahedron Lett. 21, 875–878.
Carroll, A.R., and Scheuer, P.J. (1991) J. Org. Chem. 55, 4426–4431.
Carter, D.C., Moore, R.E., Mynderse, J.S., Miemczura, W.P., and Todd, J.S. (1984) *J. Org. Chem.* 49, 236–241.

Chun, H.G., Daview, B., Hoth, D., et al. (1986) *Investigational New Drugs* 4, 279–284.

Cimino, G., Spinella, A., and Sodano, G. (1989) *Tet. Lett.* 30, 3589–3592.

Cohen, E., ed. (1979) *Biomedical Applications of the Horseshoe Crab (Limulidae)*, A.R. Liss, Inc., New York.

Cohen, S.S. (1966) in *Progress in Nucleic Acid Research and Molecular Biology*, vol. 5 (Davidson, J.N., and Cohn, W.E., eds.), pp. 1–88, Academic Press, New York.

Colwell, R.R. (1991) *Large-Scale Production of Natural Products*, National Cancer Institute Workshop, Rockville, MD, 4 Mar. 1991, National Cancer Institute, Rockville, MD.

Corey, E.J., and Carpino, P. (1989) *J. Am. Chem. Soc.* 111, 5472–5474.

Corey, E.J., Ensley, H.E., Hamberg, M., and Samuelsson, B. (1975) *J. Chem. Soc. Chem. Commun.* 277–278.

Corey, E.J., and Matsuda, P.T. (1987) *Tetrahedron Lett.* 28, 4247–4250.

Crawley, L.S. (1988) in *Pharmaceuticals and the Sea* (Jefford, C.W., Rinehart, K.L., Shield, L.S., eds.), pp. 101–107, Technomic Publishing Company, Lancaster, PA.

Crews, P., Jimenez, C., and O’Neil-Johnson, M. (1991) *Tetrahedron* 47, 3585–3600.

Crews, P., Manes, L.V., and Boehler, M. (1986) *Tetrahedron Lett.* 27, 2797–2800.

Dale, I.L., Bradshaw, T.D., Gescher, A., and Pettit, G.R. (1989) *Cancer Res.* 49, 3242–3245.

Dale, I.L., and Gescher, A. (1989) *Int. J. Cancer* 43, 158–163.

Davis, A.R., Targett, N.M., McConnell, O.J., and Young, C.M. (1989) in *Bioorganic Marine Chemistry*, vol. 3 (Scheuer, P.J.), pp. 85–114, Springer-Verlag, New York.

de Freitas, J.C., Blankemeier, L.A., and Jacobs, R.S. (1984) *Experientia* 40, 864–865.

de Silva, E.D., and Scheuer, P.J. (1980) *Tetrahedron Lett.* 21, 1611–1614.

de Silva, E.D., and Scheuer, P.J. (1981) *Tetrahedron Lett.* 22, 3147–3150.

de Souza, N.J., Ganguli, B.N., and Reden, J. (1982) in *Annual Reports in Medicinal Chemistry*, vol. 17, pp. 301–310, Academic Press, San Diego, CA.

Dorr, F.A., Kuhn, J.G., Phillips, J., and Von Hoff, D.D. (1988) *Eur. J. Cancer Clin. Oncol.* 24, 1699–1706.

Dreyfuss, M., Harri, E., Hofmann, H., et al. (1976) *Eur. J. Appl. Microbiol.* 3, 125–133.

Elyakov, G.B., Kuznetsova, T., Mikhailov, V.V., et al. (1991) *Experientia* 47, 632–633.

Ettouati, W.S., and Jacobs, R.S. (1987) *Mol. Pharmacol.* 31, 500–505.

Ewing, W.R., Bhat, K.L., and Joullie, M.M. (1986) *Tetrahedron Lett.* 42, 5863–5868.

Fattorusso, E., and Piattelli, M. (1980) in *Marine Natural Products*, vol. III (Scheuer, P.J., ed.), pp. 95–140, Academic Press, Inc., New York.

Faulkner, D.J. (1984a) *Nat. Prod. Rep.* 1, 251–280.

Faulkner, D.J. (1984b) *Nat. Prod. Rep.* 1, 551–598.

Faulkner, D.J. (1986) *Nat. Prod. Rep.* 3, 1–33.

Faulkner, D.J. (1987) *Nat. Prod. Rep.* 4, 539–576.

Faulkner, D.J. (1988) *Nat. Prod. Rep.* 5, 613–663.

Faulkner, D.J. (1990) *Nat. Prod. Rep.* 7, 269–309.

Faulkner, D.J. (1991) *Nat. Prod. Rep.* 8, 97–147.

Faulkner, D.J. (1992) *Oceanus* 35(1), 29–35.
Fenical, W. (1991) *Large-Scale Production of Natural Products*, National Cancer Institute Workshop, Rockville, MD, 4 Mar. 1991, National Cancer Institute, Rockville, MD.

Fenical, W., and Norris, J.N. (1975) *J. Phycol.* 11, 104–108.

Fimiani, V. (1987) *Oncology* 44, 42–46.

Frincke, J.M., and Faulkner, D.J. (1982) *J. Org. Chem.* 104, 265–269.

Fujiki, H., Sugunuma, M., Tahira, T., et al. (1984) in *Cellular Interactions by Environmental Tumor Promoters* (Fujiki, H., Hecker, E., Moore, R.E., Sugimura, T., and Weinstein, I.B., eds.), pp. 37–45, Japan Scientific Society's Press, Tokyo; VNU Science Press, Utrecht, The Netherlands.

Fusetani, N. (1987) in *Bioorganic Marine Chemistry*, vol. 1 (Scheuer, P.J., ed.), pp. 61–92, Springer-Verlag, New York.

Ganguly, A.K., McCombie, S.W., Cox, B., Lin, S., and McPhail, A.T. (1990) *Pure Appl. Chem.* 62, 1289–1291.

Garst, M.E., Tallman, E.A., Bonfiglio, J.N., et al. (1986) *Tetrahedron Lett.* 27, 4533–4536.

George, J.D., and George, J.J. (1979) *Marine Life*, Wiley-Interscience, New York.

Gescher, A., and Dale, I.L. (1989) *Anti-Cancer Drug Design* 48, 93–105.

Gieselman, J.A., and McConnell, O.J. (1981) *J. Chem. Ecol.* 7, 1115–1133.

Gilbert, B. (1977) *Pontif. Acad. Sci. Ser. Varia* 41, 225–252.

Glaser, K.B., De Cavalho, M.S., Jacobs, R.S., Kernan, M.R., and Faulkner, D.J. (1989) *Mol. Pharmacol.* 36, 782–788.

Glaser, K.B., and Jacobs, R.J. (1984) *Fed. Proc.* 43, 954.

Glaser, K.B., and Jacobs, R.J. (1986) *Biochem. Pharmacol.* 35, 449–453.

Glaser, K.B., Vedvick, T.S., and Jacobs, R.S. (1988) *Biochem. Pharmacol.* 37, 3639–3646.

Goodfellow, M., and Haynes, J.A. (1984) in *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (Ortiz-Ortiz, L., Nojalil, L.F., and Yakoleff, V., eds.), pp. 453–472, Academic Press, Orlando, FL.

Gregson, R.P., Marwood, J.F., and Quinn, R.J. (1979) *Tetrahedron Lett.* 4505–4506.

Gulavita, N.K., Gunasekera, S.P., Pomponi, S.A., and Robinson, E.V. (1992) *J. Org. Chem.* 1767–1772.

Gunasekera, S.P., Gunasekera, M., Longley, R.E., and Schulte, G. (1990) *J. Org. Chem.* 55, 4912–4915.

Gunasekera, S.P., Gunasekera, M., and McCarthy, P. (1991) *J. Org. Chem.* 56, 4830–4833.

Gunawardana, G.P., Koehn, F.E., Lee, A.Y., et al. (1992) *J. Org. Chem.* 57, 1523–1526.

Gunawardana, G.P., Kohmoto, S., and Burres, N.S. (1989) *Tetrahedron Lett.* 30, 4359–4362.

Gunawardana, G.P., Kohmoto, S., Gunasekera, S.P., McConnell, O.J., and Koehn, F.E. (1988) *J. Am. Chem. Soc.* 110, 4856–4858.

Guyton, A.C. (1991) in *Medical Physiology*, pp. 810–938, W.B. Saunders Company, Philadelphia, PA.

Gustafson, K., Roman, M., and Fenical, W. (1989) *J. Am. Chem. Soc.* 111, 7519–7524.

Haddad, L.M., Lee, R.F., McConnell, O.J., and Targett, N.M. (1983) in *Poisoning and Drug Overdose* (Haddad, L.M., and Winchester, J.F., eds.), pp. 303–317, W.B. Saunders Company, Philadelphia, PA.
Hall, M.J. (1989) *Biotechnology* 7(5), 427–430.

Hall, S., and Strichartz, G., eds. (1990) *Marine Toxins: Origin, Structure and Molecular Pharmacology*, American Chemical Society, Washington, DC.

Hall, S., Strichartz, G., Moczydlowski, E., Ravindran, A., and Reichardt, P.B. (1990) in *Marine Toxins* (Hall, S., and Strichartz, G., eds.), pp. 29–65, American Chemical Society, Washington, DC.

Hay, M.E., Duffy, J.E., Pfister, C.A., and Fenical, W. (1987) *Ecology* 68, 1567–1580.

Hay, M.E., and Fenical, W. (1988) *Annu. Rev. Ecol. Syst.* 19, 111–145.

Haystead, T.A.J., Sim, A.T.R., Carling, D., et al. (1989) *Nature* 337, 78–81.

Hong, C.Y., and Kishi, Y. (1990) *J. Org. Chem.* 55, 4242–4245.

Hong, C.Y., and Kishi, Y. (1991) *J. Am. Chem. Soc.* 113, 9693–9694.

Hu, S.L., and Kao, C.Y. (1985) *Toxicon* 23, 723–724.

Iguchi, K., Kaneta, S., Mori, K., et al. (1985) *Tetrahedron Lett.* 26, 5787–5790.

Ireland, C.M., Roll, D.M., Molinski, T.F., et al. (1988) in *Biomedical Importance of Marine Organisms* (Fautin, D.G., ed.), pp. 41–58, California Academy of Sciences, San Francisco.

Isenberg, H.D., and Balows, A. (1981) in *The Prokaryotes*, vol. 1 (Starr, M.P., Stolp, H., Truper, H.G., Balows, A., and Schlegel, H.G., eds.), pp. 83–122, Springer-Verlag, New York.

Isobe, M., Ichikawa, Y., Bai, D., Masaki, H., and Goto, T. (1987) *Tetrahedron* 43, 4767–4776.

Ito, S., and Hirata, Y. (1977) *Bull. Chem. Soc. Jpn.* 50, 1813–1820.

Jacobs, R.S., Culver, P., Langdon, R., O'Brien, T., and White, S. (1985) *Tetrahedron* 41, 981–984.

Jacobs, R.S., White, S., and Wilson, L. (1981) *Fed. Proc.* 40, 26–29.

Jacobs, R.S., and Wilson, L. (1986) in *Modern Analysis of Antibiotics* (Aszalos, A., ed.), pp. 481–493, Marcel Dekker, Inc., New York.

Jacobson, P.B., and Jacobs, R.S. (1991) *Fed. Proc.* 805, A510.

Jacobson, P.B., Marshall, L.A., Sung, A., and Jacobs, R.S. (1990) *Biochem. Pharmacol.* 39, 1557–1564.

Johnson, R.K., and Hertzberg, R.P. (1989) in *Annual Reports in Medicinal Chemistry*, vol. 25 (Bristol, J.A., ed.), pp. 129–140, Academic Press, San Diego, CA.

Jomon, K., Kuroda, Y., Ajsaka, M., and Sakai, H. (1972) *J. Antibiot.* 25, 271–280.

Jones, T.K., Reamer, R.A., Desmond, R., and Mills, S.G. (1990) *J. Am. Chem. Soc.* 112, 2998–3017.

Kaiser, A.B. (1988) *Am. J. Surgery* 155, 52–55.

Kao, C.Y. (1966) *Pharmacol. Rev.* 18, 997–1049.

Kato, Y., Fusetani, N., Matsunaga, S., and Hashimoto, K. (1986) *J. Am. Chem. Soc.* 108, 2780–2781.

Kato, Y., Fusetani, N., Matsunaga, S., Hashimoto, K., and Koseki, K. (1988) *J. Org. Chem.* 53, 3930–3932.

Katsumura, S., Fujiiwara, S., and Isaoe, S. (1985) *Tetrahedron Lett.* 26, 5827–5830.

Kernan, M.R., Faulkner, D.J., and Jacobs, R.S. (1987) *J. Org. Chem.* 52, 3081–3083.

Kikuchi, H., Tsukitani, Y., Iguchi, K., and Yamada, Y. (1982) *Tetrahedron Lett.* 23, 5171–5174.

Kikuchi, H., Tsukitani, Y., Iguchi, K., and Yamada, Y. (1983) *Tetrahedron Lett.* 24, 1549–1552.

Kim, S. (1967) *New Med. J. (Korea)* 10, 56–58.

Kino, T., Hatanaka, H., Hashimoto, M., et al. (1987a) *J. Antibiot.* 40, 1249–1255.
References 169

Kino, T., Hatanaka, H., Miyata, S., et al. (1987b) J. Antibiot. 40, 1256–1265.
Kitagawa, I., Hayashi, K., and Kobayashi, M. (1989) Chem. Pharm. Bull. 37, 849–851.
Kobayashi, J. (1989) J. Nat. Prods. 52(2), 225–238.
Kobayashi, M., Yasuzawa, T., Yoshihara, M., et al. (1982) Tetrahedron Lett. 23, 5331–5334.
Koehn, F.E., Longley, R.E., and Reed, J.K. (1992) J. Nat. Prods. 55, 613–619.
Komoda, Y., Kanayasu, T., and Ishikawa, M. (1979) Chem. Pharm. Bull. 27, 2491–2494.
Kraft, A.S., Smith, J.B., and Berkow, R.L. (1986) Proc. Natl. Acad. Sci. USA 83, 1334–1338.
Krebs, H.C. (1986) in Progress in the Chemistry of Organic Natural Products, vol. 49 (Herz, W., Grisebach, H., Kirby, G.W., and Tamm, C., eds.), pp. 152–400, Springer-Verlag, New York.
Larson, E.R., and Fischer, P.H. (1989) in Annual Reports in Medicinal Chemistry, vol. 24 (Allen, R., ed.), pp. 121–128, Academic Press, San Diego, CA.
Lawson, M.P., Berquist, P.R., and Cambie, R.C. (1984) Biochem. Syst. Ecol. 12, 375–393.
Leaf, A. (1988) in Pharmaceuticals and the Sea (Jefford, C.W., Rinehart, K.L., and Shield, L.S., eds.), pp. 17–22, Technomic Publishing Company, Lancaster, PA.
Lee, G.C.M. (1990a) European patent application no. 372940 A2.
Lee, G.C.M. (1990b) European patent application no. 372941 A2.
LeGrue, S.J., Sheu, T., Carson, D.D., Laidlaw, J.L., and Sanduja, S.K. (1988) Lymphokine Res. 7, 21–29.
Levine, L., and Fujiki, H. (1985) Carcinogenesis (London) 6, 1631–1634.
Levine, L., Xiao, D.-M., and Fujiki, H. (1986) Carcinogenesis (London) 7, 99–103.
Liaaen-Jensen, S., Renstrom, B., Ramdahl, T., Hallenstvet, M. (1982) Biochem. Syst. Ecol. 10, 167–174.
Lister, M.D., Glaser, K.B., Ulevitch, R.J., and Dennis, E.A. (1989) J. Biol. Chem. 264, 8520–8528.
Lombardo, D., and Dennis, E.A. (1985) J. Biol. Chem. 260, 7234–7240.
Longley, R.E., Caddigan, D., Harmody, D., Gunasekera, M., and Gunasekera, S.P. (1991a) Transplantation 52, 650–656.
Longley, R.E., Caddigan, D., Harmody, D., Gunasekera, M., and Gunasekera, S.P. (1991b) Transplantation 52, 656–661.
Longley, R.E., and Koehn, F.E. (1991) Fed. Proc. 5, 1206.
Longley, R.E., Koehn, F., Reilly, B., and Ward, J. (1993) J. Immunol. 150(2), 1594.
Look, S.A., Fenical, W., Jacobs, R.S., and Clardy, J. (1986a) Proc. Natl. Acad. Sci. USA 83, 6238–6240.
Look, S.A., Fenical, W., Matsumoto, G.K., and Clardy, J. (1986b) J. Org. Chem. 51, 5140–5145.
Malek, T.R., Ashwell, J.D., Germain, R.N., Shevach, E.M., and Miller, J. (1986) Immunol. Rev. 92, 81–87.
Manes, L.V., Naylor, S., and Crews, P. (1985) J. Org. Chem. 50, 284–286.
Marner, F.J., Moore, R.E., Hirotsu, K., and Clardy, J. (1977) J. Org. Chem. 42, 2815–2819.
Matsunaga, S., and Fusetani, N. (1991) Tetrahedron Lett. 32, 5605–5606.
Matsunaga, S., Fusetani, N., and Konosu, S. (1985a) Tetrahedron Lett. 26, 855–856.
Matsunaga, S., Fusetani, N., and Konosu, S. (1985b) J. Nat. Prods. 48, 236–241.
The Discovery of Marine Natural Products with Therapeutic Potential

Matsunaga, S., Fujicki, H., and Sakata, D. (1991) *Tetrahedron* 47, 2999–3006.

Maullem, S., Sachs, G., and Wheeler, L. (1989) U.S. patent no. 483985.

May, W.S., Sharkis, S.J., Esa, A.H., et al. (1987) *Proc. Natl. Acad. Sci. USA* 84, 8483–8487.

Mayer, A.M., Glaser, K.B., and Jacobs, R.S. (1988) *Biochem. Pharmacol.* 37, 871–878.

Mayer, A.M., and Jacobs, R.S. (1988) in *Biomedical Importance of Marine Organisms* (Fautin, D.G., ed.), pp. 133–142, California Academy of Sciences, San Francisco.

McCaffrey, E.J., and Endean, R. (1985) *Mar. Biol.* 89, 1–8.

McConnell, O.J., Hughes, P.A., Targett, N.M., and Daley, J. (1982) *J. Chem. Ecol.* 8, 1437–1453.

McGeer, E.G., Olney, J.W., and McGeer, P.L., eds. (1978) *Kainic Acid as a Tool in Neurobiology*, Raven Press, New York.

Metcalfe, S.M., and Richards, F.M. (1990) *Transplantation* 49, 798–802.

Miller, J.W., ed. (1979) *NOAA Diving Manual*, 2nd ed., pp. 11–11-13, Appendices D and E, U.S. Government Printing Office, Washington, DC.

Montgomery, D.W., Celniker, A., and Zukoski, C.F. (1987a) *Transplantation Proc.* 19, 1295–1296.

Montgomery, D.W., Celniker, A., and Zukoski, C.F. (1987b) *Transplantation* 43, 133–139.

Montgomery, D.W., and Zukoski, C.F. (1985) *Transplantation* 40, 49–56.

Moore, R.E., and Bartolini, G. (1981) *J. Am. Chem. Soc.* 103, 2491–2494.

Moore, R.E., Blackman, A.J., Cheuk, C.E., et al. (1984) *J. Org. Chem.* 49, 2484–2489.

Moore, R.E., and Entzeroth, M. (1988) *Phytochemistry* 27, 3101–3103.

Moore, R.E., Patterson, G.M.L. (1988) in *Pharmaceuticals and the Sea* (Jefford, C.W., Rinehart, K.L., and Shield, L.S., eds.), pp. 95–100, Technomic Publishing Company, Inc., Lancaster, PA.

Moore, R.E., Patterson, G.M., Carmichael, W.W. (1988) in *Biomedical Importance of Marine Organisms* (Fautin, D.G., ed.), pp. 143–150, California Academy of Sciences, San Francisco.

Munro, M.H.G., Luibrand, R.T., and Blunt, J.W. (1987) in *Bioorganic Marine Chemistry*, vol. 1, pp. 93–176, Springer-Verlag, New York.

Murata, M., Legrando, A.M., Ishibashi, Y., Fukui, M., and Yasumoto, T. (1990) *J. Am. Chem. Soc.* 112, 4380–4386.

Murata, M., Shimatani, M., Sugitani, H., Oshima, Y., and Yasumoto, T. (1982) *Bull. Jpn. Soc. Sci. Fish.* 48, 549–552.

Mynderse, J.S., Hunt, A.H., and Moore, R.E. (1988) *J. Nat. Prod.* 51, 1299–1301.

Nakamura, H., Iitaka, H., Kitahara, T., Okazaki, T., and Okami, Y. (1977) *J. Antibiot.* 30, 714–717.

Nakanishi, K., Goto, T., Ito, S., Naton, S., and Nozoe, S., eds. (1975) *Natural Products Chemistry*, vol. 2, pp. 457–463, Academic Press, New York.

Nakatsuka, M., Ragan, J.A., Sammakia, T., et al. (1990) *J. Am. Chem. Soc.* 112, 5583–5601.

Niel, G.L. (1988) in *Pharmaceuticals and the Sea* (Jefford, C.W., Rinehart, K.L., and Shield, L.S., eds.), pp. 45–48, Technomic Publishing Company, Inc., Lancaster, PA.

Nisbet, L.J., and Porter, N. (1989) *Symp. Soc. General Microbiol.* 44, 309–342.

Nonomura, H., and Ohara, Y. (1969) *J. Ferment. Technol.* 47, 463–469.
Norris, J.N., and Fenical, W. (1985) in *Handbook of Phycological Methods* (Littler, M.M., and Littler, D.S., eds.), pp. 121–144, Cambridge University Press, Cambridge, U.K.

Okami, Y. (1988) in *Horizon of Antibiotic Research* (Davis, B.D., Ichikawa, T., Maeda, K., and Midscher, L.S., eds.), pp. 213–227, Japan Antibiotic Research Association, Act Research Corporation, Tokyo, Japan.

Okami, Y., Hotta, K., Yoshida, M., et al. (1979) *J. Antibiot.* 32, 964–966.

Ouchi, K., Hiratsawa, N., Takahashi, C., et al. (1986) *Biochim. Biophys. Acta* 887, 94–99.

Paul, V.J. (1988) in *Biomedical Importance of Marine Organisms* (Fautin, D.G., ed.), pp. 23–27, California Academy of Sciences, San Francisco.

Paul, V.J., and Fenical, W. (1987) in *Bioorganic Marine Chemistry*, vol. I (Scheurer, P.J., ed.), pp. 1–29, Springer-Verlag, New York.

Paul, V.J., Hay, M.E., Duffy, J.E., Fenical, W., and Gustafson, K. (1987) *J. Exp. Marine Biol. Ecol.* 114, 249–260.

Perry, N.B., Blunt, J.W., Munro, M.H.G., and Pannell, L.K. (1988) *J. Am. Chem. Soc.* 110, 4850–4851.

Perry, N.B., Blunt, J.W., Munro, M.H.G., and Thompson, A.M.J. (1990) *J. Org. Chem.* 55, 4242–4245.

Persinos, G.J. (1989a) *Washington Insight*, March 15, North Bethesda, MD.

Persinos, G.J. (1989b) *Washington Insight*, September 15, North Bethesda, MD.

Persinos, G.J. (1989c) *Washington Insight*, December 15, North Bethesda, MD.

Persinos, G.J. (1990a) *Washington Insight*, March 15, North Bethesda, MD.

Persinos, G.J. (1990b) *Washington Insight*, June 15, North Bethesda, MD.

Persinos, G.J. (1990c) *Washington Insight*, September 15, North Bethesda, MD.

Persinos, G.J. (1990d) *Washington Insight*, December 15, North Bethesda, MD.

Persinos, G.J. (1991a) *Washington Insight*, March 15, North Bethesda, MD.

Persinos, G.J. (1991b) *Washington Insight*, September 15, North Bethesda, MD.

Persinos, G.J. (1991c) *Washington Insight*, December 15, North Bethesda, MD.

Pettit, G.R., Herald, C.L., Doubek, D.L., et al. (1982) *J. Am. Chem. Soc.* 104, 6846–6848.

Pettit, G.R., Kamano, Y., Aoyagi, R., et al. (1985) *Tetrahedron* 41, 985–994.

Pettit, G.R., Kamano, Y., Herald, C.L., Schmidt, J.M., and Zubrod, C.G. (1986a) *Pure Appl. Chem.* 58, 415–421.

Pettit, G.R., Leet, J.E., Herald, C.L., Kamano, Y., Doubek, D.L. (1986b) *J. Nat. Prods.* 49, 231–235.

*Physicians Desk Reference* (1991) Medical Economics Company, Inc., Oradell, NJ.

Poch, G.K., and Gloer, J.B. (1989a) *Tetrahedron Lett.* 30, 3483–3486.

Poch, G.K., and Gloer, J.B. (1989b) *J. Nat. Prod.* 52, 257–260.

Pomponi, S.A. (1988) in *Biomedical Importance of Marine Organisms* (Fautin, D.G., ed.), pp. 7–11, California Academy of Sciences, San Francisco.

Pomponi, S.A., Wright, A.E., Diaz, M.C., and Van Soest, R.W.M. (1991) in *Fossil and Recent Sponges* (Reitner, J., and Keupp, H., eds.), pp. 150–158, Springer-Verlag, New York.

Pulitzer-Finali, G. (1986) in *Annali del Museo Civico di Storia Naturale di Genova*, V. LXXXVI, Monotipia Erredi, Genova, Italy.

Reed, J.K., and Pomponi, S.A. (1989) in *Proceedings of the American Academy of Underwater Sciences* (Lang, M.A., and Jaap, W.C., eds.), pp. 273–287, American Academy of Underwater Sciences, Costa Mesa, CA.
Reuben, B.G., and Wittcoff, H.A. (1989) *Pharmaceutical Chemicals in Perspective*, John Wiley & Sons, New York.

Reynolds, L.J., Morgan, B.P., Hite, G.A., Mihelich, E.D., and Dennis, E.A. (1988) *J. Am. Chem. Soc.* 110, 5172–5177.

Ricklefs, R.E. (1973) *Ecology*, Chiron Press, Inc., Newton, MA.

Rinehart, K.L. (1988a) in *Pharmaceuticals and the Sea* (Jefford, C.W., Rinehart, K.L., and Shield, L.S., eds.), pp. 3–15, Technomic Publishing Company, Lancaster, PA.

Rinehart, K.L. (1988b) in *Biomedical Importance of Marine Organisms* (Fautin, D.G., ed.), pp. 13–22, California Academy of Sciences, San Francisco.

Rinehart, Jr., K.L., Gloer, J.B., Hughes, Jr., R.G., et al. (1981b) *Science* 212, 933–935.

Rinehart, Jr., K.L., Gloer, J.B., and Cook, Jr., C. (1981c) *J. Am. Chem. Soc.* 103, 1857–1859.

Rinehart, K.L., Kishmore, V., Bible, K.C., et al. (1988) *J. Nat. Prod.* 512, 1–21.

Rinehart, Jr., K.L., Shaw, P.D., Shield, L.S., et al. (1981a) *Pure Appl. Chem.* 309–385.

Rinehart, K., and Shield, L.S. (1988) in *Horizons on Antibiotic Research* (Davis, B.D., Ichikawa, T., Maeda, K., and Midscher, L.A., eds.), pp. 194–212, Japan Antibiotics Research Association, Act Japan Corporation, Tokyo, Japan.

Roth, J., LeRoith, D., Collier, E.S., Watkinson, A., and Lesniak, M.A. (1986) *Ann. N.Y. Acad. Sci.* 463, 1–11.

Roussis, V., Wu, Z., Fenical, W., et al. (1990) *J. Org. Chem.* 55, 4916–4922.

Russell, D.H., Buckley, A.R., Montgomery, D.W., et al. (1987) *J. Immunol.* 138, 276–284.

Sakemi, S., Ichiba, T., Kohmoto, S., and Saucy, G. (1988) *J. Am. Chem. Soc.* 110, 4851–4853.

Sato, K., Okazaki, T., Maeda, K., and Okami, Y. (1978) *J. Antibiot.* 31, 632–635.

Schaufelberger, D.E., Koleck, M.P., Beutler, J.A., et al. (1991) *J. Nat. Prods.* 54, 1265–1270.

Schneider, W.P., Bundy, G.L., Lincoln, F.H., Daniels, E.G., and Pike, J.E. (1977a) *J. Am. Chem. Soc.* 99, 1222–1232.

Schneider, W.P., Hamilton, R.D., and Rhuland, L.E. (1972) *J. Am. Chem. Soc.* 94, 2122–2123.

Schneider, W.P., Morge, R.A., and Henson, B.E. (1977b) *J. Am. Chem. Soc.* 99, 6062–6066.

Scheuer, P.J. (1973) *Chemistry of Marine Natural Products*, Academic Press, New York.

Scheuer, P.J. (1988) in *Biomedical Importance of Marine Organisms* (Fautin, D.G., ed.), pp. 37–40, California Academy of Sciences, San Francisco.

Scheuer, P.J., Takashi, W., Tsutsumi, J., and Yoshida, T. (1967) *Science* 155, 1267.

Schindler, L.W. (1990) *Understanding the Immune System*, NIH Publication no. 90-529, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD.

Scrip (1991) *Cancer Chemotherapy Report*, Pharmabooks, Ltd., New York.

Shin, J., and Fenical, W. (1991) *J. Org. Chem.* 56, 3153–3158.

Shimizu, Y., Bando, H., Chou, H.-N., van Duyne, G., and Clardy, J.C. (1986) *J. Chem. Soc., Chem. Commun.* 1656–1658.

Shimizu, Y., and Kamiya, H. (1983) *Marine Natural Products*, vol. V (Scheuer, P.J.), pp. 391–427, Academic Press, New York.
Stevens, D.W., Jensen, R.M., and Stevens, L.E. (1989) Transplantation Proc. 21, 1139–1140.
Stierle, A.C., Cardellina, J.G., and Singleton, F.L. (1988) Experientia 44, 1021.
Suffness, M., Newman, D.J., and Snader, K. (1989) in Bioorganic Marine Chemistry, vol. 3 (Scheuer, P.J., ed.), pp. 131–168, Springer-Verlag, New York.
Suganuma, M., Fujiki, H., Furuya-Suguri, H., et al. (1990) Cancer Res. 50, 3521–3525.
Sun, H.H., and Sakemi, S. (1991) J. Org. Chem. 56, 4307–4308.
Tachibana, K., Scheuer, P.J., Tsukitani, Y., et al. (1981) J. Am. Chem. Soc. 103, 2469–2471.
Takahashi, A., Nakamura, H., Kameyama, T., et al. (1987) J. Antibiot. 40, 1671–1676.
Tamplin, M.L. (1990) in Marine Toxins (Hall, S., and Strichartz, G., eds.), pp. 78–86, American Chemical Society, Washington, DC.
Technical Resources, Inc. (1987) in Workshop on Dereplication of Bioactive Natural Products, May 28–29, 1987, Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.
Torres, J.C. (1988) in Pharmaceuticals and the Sea (Jefford, C.W., Rinehart, K.L., and Shields, L.S., eds.), pp. 109–112, Technomic Publishing Company, Lancaster, PA.
Trainer, V.L., Edwards, R.A., Szment, A.M., et al. (1990) in Marine Toxins (Hall, W., and Strichartz, G., eds.), pp. 166–175, American Chemical Society, Washington, DC.
Trenn, G., Pettit, G.R., Takayama, H., Hu-Li, J., and Sitkovsky, M.V. (1988). J. Immunology 140, 433–439.
Tullar, R.M. (1972) Life: Conquest of Energy, Holt, Rinehart and Winston, Inc., New York.
Uemura, D., Ueda, K., Hirata, Y., Naoki, H., and Iwashita, T. (1981) Tetrahedron Lett. 22, 2781–2784.
Umezawa, H., Okami, Y., Kurasawa, Y., et al. (1983) J. Antibiot. 36, 471–477.
Van Soest, R.W.M., and Stentoft, N. (1988) Studies Fauna Curacao Other Caribbean Islands 70, 1–175.
Waldman, T.A. (1986) Science 232, 727–729.
Wattenberg, E.V., Fujiki, H., and Rosner, M.R. (1987) Cancer Res. 47, 4618–4622.
Weed, S.D., and Stringfellow, D.A. (1983) Antiviral Res. 3, 269–274.
Weinheimer, A.J., and Spraggins, R.L. (1969) Tetrahedron Lett. 5185–5188.
Weyland, H. (1969) Nature 223, 858.
Wheeler, L.A., Sachs, G., De Vries, G., et al. (1987) J. Biol. Chem. 262, 6531–6538.
Whittaker, R.H., and Feeny, P.P. (1971) Science 171, 757–770.
Wilkinson, S.R. (1978) Marine Biol. 49, 161–167.
Wilkinson, S.R. (1980) in Endocytobiology, Endosymbiosis and Cell Biology, vol. 1 (Schwemmler, W., and Schenk, H.E.A., eds.), pp. 553–563, Walter de Gruyter, New York.
Williams, L. I., Stone, M.J., Hauck, P.R., and Rahman, S.K. (1989) J. Nat. Prod. 52(6), 1189–1208.
Winston, J. E. (1988) in Biomedical Importance of Marine Organisms (Fautin, D.G., ed.), pp. 1–6, California Academy of Sciences, San Francisco.
Wratten, S.J., Wolfe, M.S., Andersen, R.J., and Faulkner, D.J. (1977) Antimicrob. Agents Chemother. 11, 411–414.
Wright, A.E., Forleo, D.A., Gunawardana, G.P., et al. (1990) J. Org. Chem. 55, 4512–4515.
Yasumoto, T., and Murata, M. (1990) in Marine Toxins (Hall, S., and Strichartz, G., eds.), pp. 120–132, American Chemical Society, Washington, DC.
Yasumoto, T., Nakamjima, I., Bagnis, R., and Adachi, R. (1977) Bull. Jpn. Soc. Sci. 43, 1021–1026.
Yuh, D.D., Zurcher, R.P., Carmichael, P.G., and Morris, R.E. (1989) Transplantation Proc. 21, 1141–1143.
Zobell, C.E. (1941) J. Marine Res. 4, 42–75.