Bioactive substances from aquatic sources

Suseela Mathew
Biochemistry and Nutrition Division
Central Institute of Fisheries Technology
Cochin-682029

Natural products have long been used as foods, fragrances, pigments, insecticides, medicines, etc. Due to their easy accessibility, terrestrial plants have served as the major source of medicinally useful products, especially for traditional or folk medicine. About 25% of all pharmaceutical sales are drugs derived from natural plant products and an additional 12% are from microbial source. The marine environment covers a wide thermal range (from the below freezing temperatures in Antarctic waters to about 350°C in deep hydrothermal vents), pressure range (1-1000 atm), nutrient range (oligotrophic to eutrophic) and it has wide ranging photic and non-photic zones. This extensive variability has facilitated extensive speciation at all phylogenetic levels, from microorganisms to mammals. Despite the fact that the bio diversity in the marine environment far exceeds that of the terrestrial environment, research into the use of marine natural products as pharmaceutical agents is still in its infancy. This may be due to the lack of ethno-medical history and the difficulties involved in the collection of marine organisms. But with the development of new techniques, it is possible to collect marine samples and during the past decade, over 5000 novel compounds have been isolated from marine sources.

The biomedical potential offered by marine organisms

In order to evaluate the biomedical potential of any plant or animal, one must consider both the chemical ecology of the organism and its evolutionary history. Chemical defense mechanisms evolved with the most primitive microorganisms have been replaced in advanced organisms by physical defenses and/or the ability to run or swim away and hide. Sessile, soft-bodied marine invertebrates that lack obvious physical defenses are therefore prime candidates to possess bioactive metabolites. Sessile marine invertebrates have a very long evolutionary history and have had ample opportunity to perfect their chemical defenses. Chemical defense mechanisms cannot be directly equated with potential biomedical activity, but it is remarkable how well the two correlate in reality. This could be explained by the fact that targets of the chemical defenses, primary metabolites such as enzymes and receptors are highly conserved compared with secondary metabolites. Among the many phyla found in the oceans, the best sources of pharmacologically active compounds are bacteria (including cyanobacteria), fungi, certain groups of algae, sponges, soft corals, gorgonians, sea hares, nudibranchs, bryozoans, mollusks and tunicates. Some

marine organisms such as dinoflagellates, echinoderms and some fish, are well known for their ability to produce potent toxins, but these are usually too toxic for medicinal use.

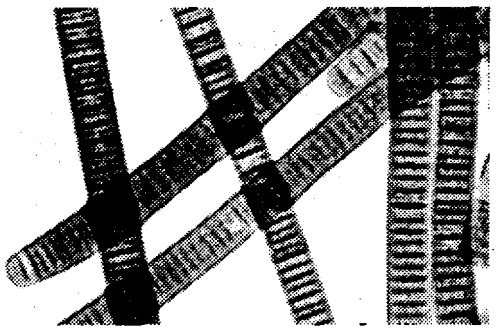
Screening of marine extracts

The process of discovering marine pharmaceuticals starts with the collection of marine organisms. This is often the most important step in the entire research program because the quality of the collections influences all future research. For those who are unfamiliar with scientific collecting, it must seem terribly romantic to travel to distant coral reefs to dive for new specimens. In reality, collecting expeditions are far from romantic. The divers must find sites that offer a combination of high biological diversity and density and the collection of specimens should not adversely impact the collection site. The collection must be accurately sorted and carefully documented so that the specimens can be re-collected in the future. After a sub-sample has been put aside for taxonomic studies, the bulk sample must be rapidly frozen or stored in solvent to retard bacterial degradation of the specimens. Crude extracts are then prepared. It is at this step that a crucial decision must be made concerning the screening strategy to be adopted, since this defines the biomedical utility of the research program. Fortunately, no two research groups have exactly the same screening strategy, but it is worth reviewing some representative strategies. The pharmaceutical industry is now almost totally dependent on high-throughput screens that employ robots to perform bioassays using a 96-well plate format. This regime was designed to handle large libraries of pure compounds but, due to problems involving solubility and/or non-specific inhibition, may not provide optimal results for crude extracts. Since high-throughput screens are usually run for a limited length of time, the natural products chemist may be at a disadvantage when a bioassay-guided fractionation takes longer to complete than the screening of the pure chemical library. Yet the natural products often turn out to be the more active 'hits'. This raises the issue of quantity *vs.* quality, which every group must address in their own way. With the exception of those studying marine bacteria and fungi, the average academic marine natural products program collects 200–400 new marine organisms per year, which is clearly not enough samples to justify the creation of high-throughput screens in house. One strategy is to send crude extracts to many academic collaborators, each of whom has a specialized research interest. Assays that require careful interpretation but provide a lot of information per assay are ideal for marine natural products research, although they are more difficult to use during a bioassay-guided fractionation. Since crude extracts are not well tolerated by some bioassays, one may randomly isolate pure compounds on the basis of interesting chemical structures and screen libraries of pure compounds. This strategy puts marine natural products on the same footing as synthetic compounds in high-throughput screens but it requires great patience to amass a good library of compounds.

Marine Bacteria as a Source of Metabolites

In the past century, microorganisms had played an important role in the production of antibiotics and other drugs for the

treatment of some serious diseases. Since the discovery of penicillin in 1929 to the Taq DNA polymerase obtained from *Thermus aquaticus* (Yellowstone hot spring) in 1989, nearly 50,000 natural products have been discovered from microorganisms. Over 10,000 of these are reported to have biological activity and over 100 microbial products are in use today as antibiotics, antitumour agents, and agrochemicals. In spite of such successes in drug discovery from microorganisms, marine microorganisms have received very little attention. The difficulty in the search of metabolites from marine bacteria is mainly due to the non-culturability of the majority (over 99%). Marine bacteria are capable of producing unusual bioactive compounds that are not observed in terrestrial sources. Thermo-stable proteases, lipases, esterases, and starch and xylan degrading enzymes have been actively sought and in many cases are found in bacterial and archaeal hyperthermophilic marine microorganisms. An unusual gram-positive bacterium from deep-sea sediment, which produced a series of new natural products, macrolactin A-F of an unprecedented C_{24} linear acetogen origin has been isolated. The major metabolite, macrolactin A inhibits B16-F10 murine melanoma cells in *in vitro* assays, showing



Oscillatoria spp.

significant inhibition of mammalian herpes simplex virus (type I and II) and protecting T lymphocytes against human immuno-deficiency virus (HIV) replication. On the other hand, a microbial metabolite with anti-HIV potential (from *Alteromonas* spp.) has been developed as reverse transcriptase inhibitor from marine microbes isolated from the tissues of Bermudian marine sponge. Some *Vibrio* species have been found to produce a variety of extra cellular proteases. *Vibrio alginolyticus* produces six proteases including an unusual detergent-resistant, alkaline serine exoprotease. This marine bacterium also produces collagenase, an enzyme with a variety of industrial and commercial applications, including the dispersion of cells in tissue culture studies.

Metabolites from Marine Cyanobacteria

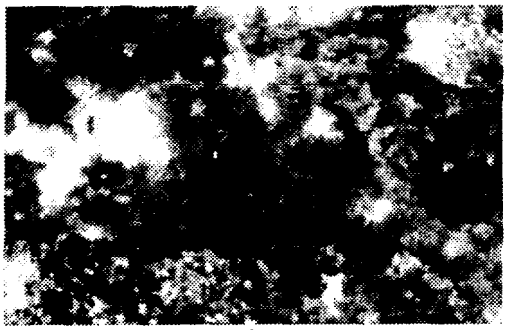
Cyanobacteria in general and marine forms in particular are one of the richest sources of known and novel bioactive compounds including toxins with wide pharmaceutical applications. Among the five divisions of microalgae, studies of biomedical natural products have been concentrated only on two divisions, i.e., Cyanophyta (blue-green algae) and Pyrrophyta (dinoflagellates). Although several metabolites have been isolated from cyanophytes, most of them are isolated from fresh water species, which are cultured easily in comparison to marine organisms. Lyngbyatoxin-A and debromoaplysiatoxin are two highly inflammatory but structurally different metabolites isolated from toxic strains of *Lyngbya mausculata* collected in Hawaii, and anatoxin-a from *Anabaena ciecinalis*. Some of the marine cyanobacteria appear to be potential sources

for large-scale production of vitamins of commercial interest such as vitamins of the B complex and vitamin-E. The carotenoids and phycobiliprotein pigments of cyanobacteria have commercial value as natural food colouring agents, as feed additives, as enhancers of the color of egg yolks, to improve the health and fertility of cattle, as drugs and in the cosmetic industries. Some anti-HIV activity has been observed with the compounds extracted from *Lyngbya lagerhaimanii* and *Phormidium tenue*. Cultured *Fusarium chlamydosporum* isolated from the Japanese marine red alga *Carpopeltis affinis* is the source of fusaperazines A & B, two new sulphur containing dioxopiperazine derivatives, and two known compounds which had been originally isolated from the fermentation by the fungus *Tolypocladium* spp. Chalcomycin-B exhibited activity against a variety of microorganisms and microalgae. Four new epipolysulphanyldioxopiperazines were isolated from a culture of the fungus *Leptosphaeria* spp. originating from the Japanese brown alga *Sargassum tortile*. Each compound possessed significant cytotoxic activity against the P388 cell-line, while one of the leptosins also exhibited appreciable cytotoxicity against a disease-oriented panel of 39 human cancer cell-lines, and specifically inhibited two protein kinases and topoisomerase-II. Cultures of the marine fungus *Hypoxylon oceanicum* from mangrove wood at Shenzen, China, yielded the macrocyclic polyesters and the linear polyesters. The compounds exhibited modest activity against the phytopathogenic fungus *Neurospora crassa*.

The anti-inflammatory and anti-proliferative properties of scytonemin, an extracellular sheath pigment originally isolated from the cyanobacterium *Stigonema* spp. have been reported. Goniodomin-A, an antifungal polyether macrolide from the dinoflagellate *Goniodoma pseudogoniaulax* has been shown to inhibit angiogenesis by the inhibition of endothelial cell migration and basic fibroblast growth factor (bFGF)-induced tube formation and is active *in vivo*. An immunosuppressive linear peptide microcolin-A, which at nanomolar concentrations suppresses the two way murine mixed lymphocyte reaction, has been isolated from *Lyngbya majusculata*. A unique thiozoline-containing compound, curacin- A, has been purified from the organic extract of a Curacao collection of *L. majusculata*. This compound has been found to be an exceptionally potent antiproliferative agent as it inhibits the polymerization of tubulin, which shows some selectivity for colon, renal and breast cancer-derived cell lines. A series of noval antibiotics agents have been isolated from dianoflagellates, antifungal agents from *Gambierdiscus toxicus* and brevitoxins from *Ptychodiscus brevis*. Okadaic acid, a polyether fatty acid produced by *Prorocentrum* spp., has been a key molecule in studying signal transduction pathways in eukaryotic cells since it is a selective protein phosphatase inhibitor.

Metabolites from seaweeds

Seaweeds are abundant in the inter-tidal zones and in clear tropical waters. Presently the seaweed industry consists of two kelps, three *Gelidium* species one *Gracilaria-/Gracilariopsis* species, etc. In addition, there are a number of seaweeds with economic potential. The red algae *Sphaerococcus coronopifolius* was shown



Ulva fasciata

to have antibacterial activity ; the green alga *Ulva lactuca* was shown to posses an anti-inflammatory compound; and an anti-tumor compound was isolated from *Portieria hornemannii. Ulva fasciata* produces a novel sphingosine derivative with antiviral activity *in vivo*. A cytotoxic metabolite, stypoldione, which inhibits microtubule polymerization and thereby prevents mitotic spindle formation, has been isolated

Sargassum

from tropical brown alga, *Stypodium zonale. P. hornemannii* is found to be a novel source of cytotoxic penta halogenated monoterpene, halomon, which exhibited one of the most extreme examples of differential cytotoxicity in the screening conducted by the National Cancer Institute (NCI), USA. Halomon has been selected for preclinical drug development since this compound shows toxicity to brain, renal and colon tumor cell-lines and preliminary *in vivo* evaluations have been encouraging. An iodinated nucleoside has been isolated from *Hypnea valitiae*, which is a potent and specific inhibitor of adenosine kinase. It can be used in the studies of adenosine receptors in a variety of systems, and in studies on nucleotide metabolism and regulation . The green alga *Codium iyengarii* from the Karachi coast of the Arabian Sea ia a source of a steroid, iyengadione and two new steroidal glycosides, iyengarosides A and B. Iyengaroside-A displayed moderate activity against a range of bacteria. *Sargassum carpophyllum* from the South China Sea is the source of two new bioactive sterols. These sterols induced morphological abnormality in the plant pathogenic fungus *Pyricularia oryzae*; also exhibited cytotoxic activity against several cultured cancer cell lines. *Sargassum polycystum* collected in the North China Sea yielded a new sterol, stigmast. The fact that there are many algae that can convert simple polyunsaturated fatty acids such as arachidonic acids into complex eicosanoids and related oxylipins has been an exiting development. Derivatives of arachidonic acids are important in maintaining homeostasis in mammalian systems and aberrant production of metabolites of this class occurs in diseases such as psoriasis, asthma, arteriosclerosis, heart disease, ulcers and cancer.

Metabolites from Sponges

Approximately 10,000 sponges have been described in the world and most of them live in marine waters. A range of bioactive metabolites has been found in

about 11 sponge genera. Three of these genera (*Haliclona*, *Petrosia* and *Discodemia*) produce powerful anti-cancer, antiinflammatory agents, but their cultivation has not been studied. The discovery of spongouridine, a potent tumor-inhibiting arabinosyl nucleoside in Caribbean sponge *Cryptotethia crypta*, focused attention on sponges as a source of biomedically important metabolites.

Metabolites from Cnidarians

The discovery of prostaglandin in corals in the late 1960s contributed greatly to the rapid developments in the field of marine natural products. Palytoxin, which is one of the most potent known toxins, is the product of *Palythoa* species of the family Zoanthidae. It is a useful tool for probing cellular recognition processes since it stimulates arachidonic acid metabolism and down-regulates the response to epidermal growth factor by activating a sodium pump in the signal transduction pathway using sodium as the second messenger. Bioassay-guided fractionation of extracts obtained from soft coral, *Lobophytum crassum*, indicated ceramideas a moderately antibacterial component. New examples of cadinene-skeleton sesquiterpenes, xenitorins A–F, have been isolated from *Xenia puerto-galerae*. Xentorin A and E exhibited cytotoxicity towards the A and P388 tumour cell-lines. Lophotoxin from the genus *Lophogorgia* preferentially binds to the nicotinic subunit of acetylcholine receptors and blocks out cholinergic nicotinic pathway in a complex set of interacting neurons. Pseudopetrocin-E, a tricyclic diterpene pentoside from gorgonians of the genus *Pseudopterogorgia*, shows anti-inflammatory and analgesic activities equal in potency to industrial standard indomethicine.

Metabolites from molluscs

More than 2600 scientific studies over the last 20 years testify to the important

Fasciospongia sp.

contribution of toxins extracted from cone snails to medicine and cellular biology. To date, only 100 out of a potential 50,000 toxins have been extracted and analyzed. The *Conus* species have evolved deadly nerve toxins and small, conformationally constrained peptides of 10-30 amino acids. Some of the conotoxins block channels regulating the flow of potassium or sodium across the membranes of nerve or muscle cells; others bind to N-methyl-D-aspartate receptors to allow calcium ions into nerve cells; and some are specific antagonists of acetylcholine receptors responsible for muscle contraction. Thus, conotoxin are valuable probes in physiological and pharmacological studies. Neosurugatoxin isolated from *Babylonia japonica* is useful in characterizing two classes

of acetylcholine receptors. Dolastatin, a cytotoxic peptide from *Dolabella auricularia* is an antineoplastic substance. Ulapualide-A, a sponge-derived macrolide isolated from the nudibranch *Hexabranchus sanguineus* exhibits cytotoxic activity against L 1210 murine leukemia cells and antifungal activity, which exceeds that of clinically useful amphotericin-B. Chromodorolide-A isolated from *Chromocloris cavae* exhibits *in vitro* antimicrobial and cytotoxic activities.

Metabolites from tunicates

Didemnin-B from the Caribbean tunicate *Tridemnum solidum* was the first marine compound to enter human cancer clinical trial as a purified natural product , but was unsuccessful in further trials . Nevertheless, this class of cyclic peptides provides important structural lead for a variety of antiviral, anticancer and immunosuppressant activities. The inhibitor of matrix metalloproteinase (MMP2), from an ascidian of the family Polyclinidae collected off Western Japan, was identified as sodium1- (12-hydroxy) octadecanyl sulphate. Two unusual trithiocane derivatives were isolated from the ascidian *Perophora viridis* collected off North Carolina. Both compounds exhibited mild antibacterial activity as well as toxicity towards brine shrimp. Halocidin was isolated as an antimicrobial peptide (3443 Da) from the hemocytes of the solitary ascidian *Halocynthia aurantium*. Lepadins D with an unidentified counter ion, lepadins E and lepadins F were isolated as antiplasmodial and antitrypanosomal alkaloid constituents of a *Didemnum* spp. ascidian collected from Stanley Reef, the Great Barrier Reef. Coproverdine is a cytotoxic alkaloid isolated by bioassay-directed fractionation of an unidentified ascidian collected at the Three Kings Islands, New Zealand. Ecteinascidin isolated from *Ectenascidia turbinata* shows potent activity *in vivo* against a variety of mouse tumour cells. Rubrolide-M, recently isolated from a Spanish collection of the ascidian *Synoicum blochmanni*, was synthesised using palladium catalysed coupling methodology.

Metabolites from Fish, Sea Snakes and Marine Mammals

Metabolites extracted from fish, sea snakes and aquatic mammals are scanty. Various fish species are used to extract fish oil, rich in omega-3 fatty acids, which are used in the preparation of various kinds of drugs for the remedies of human beings, such as arthritis and many other diseases. Through out the world about 500 species of fish are considered toxic. The most spectacular substance of pharmacological importance extracted from fish is tetradotoxin (TTX), the puffer or fugu poison. Other toxins isolated include ciguatoxin from electric rays, which is a potent antidote for pesticide poisoning. An anticancerous drug, namely "Fu-anntai", which has antiblastic effects on cervical carcinoma, stomach cancer, rhinocarnoma and leukemia cells, has been extracted from sea snakes in China. A group of scientists in Australia have extracted a novel drug from rat snake

Other Metabolites

Polyunsaturated Fatty Acids (PUFA)

Polyunsaturated fatty acids consist of two parent compounds: linoleic acid, a fatty acid of the n-6 family (18:2n6) and α-linolenic acid, a fatty acid of the n-3 family (18:3n3). These fatty acids have

different metabolic effects. Linoleic acid (LA) can be elongated to arachidonic acid (AA), a fatty acid with 20 carbon atoms and 4 double bonds (20:4n6) with two intermediary metabolites termed γ-linolenic acid (18:3n6, GLA) and dihomo-γ-linolenic acid (20:3n6, DHLA). α–linolenic acid can be elongated to either eicosapentaenoic acid (EPA), a fatty acid with 20 carbon atoms and 5 double bonds (20:5n3) or docosahexaenoic acid (DHA), a fatty acid with 22 carbon atoms and 6 double bonds (22:6n3). The fatty acids with 20 carbon atoms, AA and EPA play an important role in prostaglandin metabolism and may influence the thrombotic process. Various studies indicate that the intake of α-linolenic acid reduces risk of coronary heart disease. Whether this effect is independent of other unsaturated fatty acids e.g. linoleic acid, is difficult to establish because different unsaturated fatty acids are present in the same foods e.g. soybean oil. However, the hypothesis of a protective effect of α-linolenic acid in relation to coronary heart disease is supported by the results of the Lyon trial. In this intervention study, a Mediterranean diet enriched with α-linolenic acid was strongly protective in relation to coronary heart disease. However, more data are needed before definite statements can be made about the possible protective effect of α-linolenic acid.

During the past 20 years, chemists have discovered a large number of promising pharmaceuticals from marine flora and fona. It must be acknowledged that there have been difficulties in supplying sufficient materials for clinical trials, but many of the problems experienced in the past seem close to being solved. "Poison kills the poison," the famous proverb is the basis for researchers in finding the biomedical metabolites from living organisms. Sea has got plenty of metabolites and other resources in living or dead form. Sponges (37%), coelenterates (21%) and microorganisms (18%) are the major sources of biomedical compounds followed by algae (9%), echinoderms (6%), tunicates (6%), molluscs (2%) bryozoans (1%), etc. The main emphasis is given in the search of drugs for deadly human diseases as cancer and AIDS. The scientists at different parts of the world have extracted various drugs for such diseases in recent years.

Synthetic methods are constantly improving so that even complex molecules or preferably, simpler analogs based on marine metabolites, can be synthesized on industrially useful scales. Aquaculture of marine invertebrates is a reality and tissue culture of invertebrate cells shows some promise. In the future, gene transfer technology may allow even complex biosynthetic pathways to be reproduced easily in cultured bacteria.

We have a few immediate problems to solve. One is the apparent incompatibility of high-throughput screening with natural product isolation which can and must be solved if natural products are to be used to their greatest advantage. Another problem is the increasing regulation of bioprospecting research which has prevented both scientific discoveries and, in a strange twist of fate, biodiversity conservation. It now seems more obvious than ever that marine natural products should be regarded as the inspiration for new pharmaceuticals and that every effort should be made to avoid the wild harvesting

Table 1.Examples of commercialised bioactive compounds from marine organisms

Chemical name	Origin	Activity	Type of molecule	Year
Cephalosporins	*Cephalosporium* sp. (marine fungi)	antibiotic	b-lactam	1965
Cytrabine[Ara-C]	*Cryptotethya crypta* Sponge	Antimicrobial (Cytotoxic)	Nucleoside	1972
Kainic acid	*Digenea simplex* Red algae	Antihelmintic Insecticide	Amino acid	Early 1900
Spongoadenosine [Ara-A]	*Cryptotethya crypta* Sponge	Antiviral Herpes	Nucleoside	Not known
Zinconotide	*Conus mogus* Mollusc	Analgesic	Peptide	1999

of marine invertebrates to provide commercial quantities of the drugs themselves

Bibliography

Bartik K, Braekman J, C, Daloze D, Stoller C, Huysecom J, Vandevyver G & Ottinger P (1987) Topsentins, new toxic bisindole alkaloids from the marine sponge *Topsentia genitrix. Can. J. Chem.* 65: 2118-2121.

Crews P,Manes LV & Boehler M(1986) Jasplakinolide, a cyclodepsipeptide from the marine sponge *Jaspis sp. Tetrahedron Lett.* 27: 2797-2800.

de Silva ED & Scheuer PJ (1980) Manoalide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis* (Polejaeff). *Tetrahedron Lett.* 21: 1611-1614.

Faulkner DJ (1993) Academic chemistry and the discovery of bioactive marine natural products. In: Attaway DH & Zaborsky OR (Eds.). Marine Biotechnology, Vol. 1 Pharmaceutical and Bioactive Natural Products, Plenum Press, New York. pp 459-474.

Harrigan GG, YoshidaWY, Moore RE, Nagle DG, Park PU, Biggs J, Paul VJ, Mooberry SL, Corbett TH & Valeriote FA (1998) Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula/Schizothrix calcicola cyanobacterial assemblages. J. Nat.* Prod. 61: 1221-1225.

Lai J-Y, Yu J, Mekonnen B & Falck JR (1996) Synthesis of curacin A, an antimitotic cyclopropane-thiazoline from a marine cyanobacterium *Lyngbya majuscula. Tetrahedron Lett.* 37: 7167-7170.

Look SA, Fenical W, Matsumoto GK, & Clardy J (1986b) The pseudopterosins: a new class of antiinflammatory and analgesic diterpene pentosides from the marine sea whip *Pseudopterogorgia elisabethae* (Octocorallia). *J. Org.* Chem. 51: 5140-5145.

Murray LM, Lim TK, Hooper JNA & Capon RJ (1995) Isobromotopsentin: a new bis(indole) alkaloid from a deepwater marine sponge *Spongosorites* sp. *Aust. J. Chem.* 48:

2053-2058.

Ortiz AR, Pisabarro MT & Gago F (1993) Molecular model of the interaction of bee venom phopholipase A2 with manoalide. *J. Med. Chem.* 36: 1866-1879.

Pettit GR, Kamano Y, Herald CL, Tuinman AA, Boettner FE, Kizu H, Schmidt JM, Baczynskyj L, Tomer KB & Bontems R. (1987) The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. *J. Am. Chem. Soc.* 109: 6883-6885.

Potts BCM, Faulkner DJ & Jacobs RS (1992a) Phospholipase A2 inhibitors from marine organisms. *J. Nat. Prod.* 55: 1701-1717.

Rinehart KL, Holt TG, Fregeau NL, Stroh JG, Kiefer PA, Sun F, Li LH & Martin DG (1990) Ecteinascidins 729, 743, 745, 759A, 759B and 770: potent antitumor agents from the Caribbean tunicate *Ecteinascidia turbinata. J. Org. Chem.* 55: 4512-4515.

Rossi JV, Roberts MA, Yoo HD & Gerwick WH (1997) Pilot scale culture of the marine cyanobacterium *Lyngbya majuscula* for its pharmaceutically useful natural metabolite curacin *A. J. Appl. Phycol.* 9: 195-204.

Roussis R, Zhongde & WFenical, W (1990) New antiinflammatory pseudopterosins from the marine octocoral *Pseudopterogorgia elisabethae. J. Org. Chem.* 55: 4916-4922.

Sakai R, Rinehart KL, Kishore V, Kundu B, Faircloth G, Gloer JB, Carney JR, Namikoshi M, Sun F, Hughes RG Jr, Grávalos DG, de Quesada TG, Wilson GR & Heid RM (1996) Structure-activity relationships of the didemnins. *J. Med. Chem.* 39: 2819-2934.

Sigel MM, Wellham LL, Lichter W, Dudeck LE, Gargus J & Lucas AH (1969) Anticellular and antitumor activity of extracts from tropical marine invertebrates. In: Younghen HW Jr (Ed), Food-Drugs from the Sea Proceedings, pp 281-294.

Wright AE, Forleo DA, Gunawardana GP, Gunasekera SP, Koehn FE & McConnellOJ (1990) Antitumor tetrahydroisoquinoline alkaloids from the colonial ascidian *Ecteinascidia turbinata. J. Org. Chem.* 55: 4508-4512.