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MARINE PHARMACOLOGY: BIOACTIVE MOLECULES FROM THE SEA

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INTRODUCTION

Mankind has known for at least several thousand years that marine organisms contain substances capable of potent biological activity (1). Undoubtedly we are aware that most of the currently available therapeutic agents stem either directly or indirectly from naturally occurring organic molecules derived from terrestrial plants and/or animals. However, the abundant floras and faunas inhabiting the 70% of the Earth's surface covered by the ocean waters remain relatively unexplored. It is only in the past three decades that a significant research activity has suggested that the sea offers an enormous biomedical potential yet to be harnessed by man.

Attention to pharmacologically active substances in the sea in recent decades was first drawn by Emerson & Taft (2). Several subsequent reviews and monographs have dealt mostly with the pharmacology and toxicity of crude and semipurified extracts of marine organisms (3–13). More recent reviews, however, describe the pharmacology of some of the pure compounds of marine origin (14–17).

This review focuses on pharmacological activities of pure compounds isolated from marine organisms, as well as the pharmacologic activities of some highly potent substances broadly given the notorious name of marine toxins. Note that we take the same view of toxicity as Paracelsus—that at a given dosage level all compounds are toxic but that the right dose via the right route can make all the difference. Digitalis and tetrodotoxin (18) are but two examples among many that support this view.

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CARDIOVASCULAR-ACTIVE SUBSTANCES

Marine Nucleosides

When subjected to chemical and pharmacological characterizations, an asystolic factor and a cardiotonic fraction from extracts of the sponge *Dasychalina cyathina* (19) led to the isolation of adenosine (20) and 2'-deoxyadenosine (21). Both these nucleosides showed negative inotropic and chronotropic activities on the isolated perfusing guinea-pig heart, leading to a heart block and a coronary vasodilation as would be expected of adenosine (particularly the heart block) (22). Spongosine, a methoxy derivative of adenosine (Figure 1), was obtained from the extract of the Caribbean sponge, *Cryptotethia crypta,* while being studied for the isolation of another asystolic activity (23). It should be pointed out here that it was the structural modification of an unusual arabinosylnucleoside obtained from *C. crypta* (24) that led to the development of cytosine arabinoside (ara C), a clinically effective antileukemic drug of choice available today (25). Doridosine, N^1-methylisoguanosine, was isolated from the nudibranch *Anisodoris nobilis* and found to be a prolonged hypotensive agent (26).

A comparative cardiovascular study (23) of various marine nucleosides in our laboratories shows that these compounds reduce both the rate and the force of contraction of the heart; their relative potencies are adenosine > doridosine > isoguanosine > spongosine. However, their potencies in increasing the coronary flow as a result of coronary dilation are adenosine > spongosine > isoguanosine > doridosine. Caffeine, a blocker of adenosinergic receptors, blocks these effects of marine nucleosides on the force as well as rate of myocardiac contractility, but somewhat less effectively on the coronary flow. Whether or not different types of purinergic receptors are involved in mediating the cardiac muscular and vascular effects remains an open question.

The hypotensive response in anesthetized rats and guinea pigs was most pronounced in the case of doridosine, followed by spongosine and isoguanosine. This may be attributable to the relatively longer half-life of the nucleosides in question, for in vitro incubation of these compounds with adenosine deaminase revealed virtually no disappearance with time of doridosine and less than 5% decrease of spongosine concentrations (P. Kaul, unpublished). On the other hand, adenosine disappeared rapidly while isoguanosine exhibited an intermediate rate of disappearance.

Intraperitoneal (i.p.) injections of the marine nucleosides (1–50 μg/kg) in mice as well as their injection into the lateral ventricles of conscious guinea pigs produced a dose-dependent fall in body temperature (27). The rate, magnitude, and duration of hypothermia were most profound in the case of doridosine, followed by spongosine and isoguanosine. This prolonged effect may be due to

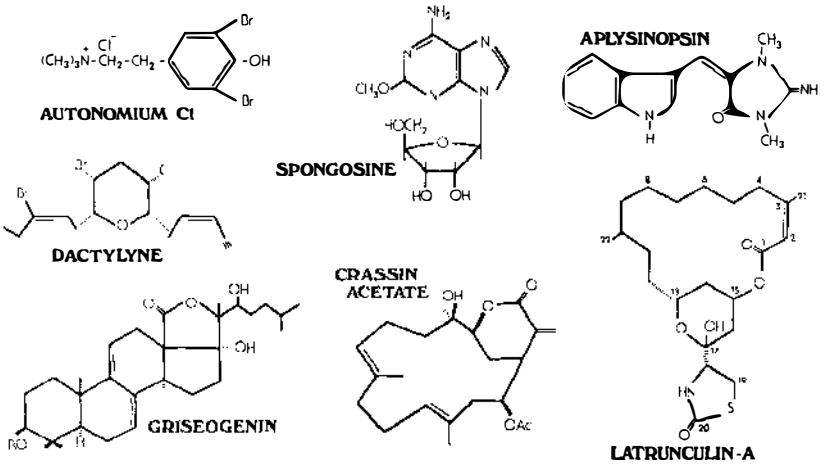

Figure 1 Various pharmacologically active molecules isolated from marine organisms.

a relatively slow disappearance of doridosine. The hypothermia was dose-dependently attenuated by pretreatment with theophylline (1–10 μg/kg i.p.). These prolonged hypotensive (26) and hypothermic (27) effects of doridosine make it an interesting candidate for further studies to determine its detailed pharmacological and toxicity profiles in order to assess its potential as a clinically useful agent.

Recently, pharmacology of two novel halogenated pyrrolopyrimidine derivatives of adenosine from marine organisms has been reported (28). 4-Amino-5-bromo-pyrrolo [2,3-d] pyrimidine from a sponge of genus *Echinodictyum* exhibited bronchodilatory activity similar to that of theophylline, but unlike theophylline it did not possess any central anti-adenosinergic activity. However, it has a potent inhibitory effect on adenosine reuptake and adenosine kinase in the brain tissue. The compound (10 or 30 mg/kg p.o.) had no significant effect on the heart rate or blood pressure of normotensive rats and only a small (about 5%) depressor effect in the desoxycorticosterone acetate (DOCA)/salt hypertensive rats.

The other nucleoside, 5'-deoxy-5-iodotubercidin, isolated from the red alga *Hypnea valentiae*, was found to be a muscle relaxant and hypothermic in mice. It was a very potent inhibitor of adenosine kinase of guinea-pig and rat brains and of rat liver. Interestingly, neither of the compounds was a substrate for or an inhibitor of adenosine deaminase. The iodotubercidin compound appears to be unique in that it is the first example of a naturally occurring 5'-deoxyribosyl nucleoside and an iodinated nucleoside (28). Because of its structural uniqueness and highly potent inhibitory activity on adenosine kinase, it may turn out to be an interesting research tool in physiology and pharmacology.

Marine Glycosides and Saponins

Terrestrial plants richly abound in glycosides and saponins, but in the animal kingdom only marine organisms have been found to contain these compounds, exclusively in the phylum *Echinodermata*. Of the five classes of this phylum, only *Holothuroidae* and *Asteroidae* contain appreciable quantities of saponins, generally referred to as holothurins and asterosaponins, respectively. The aglycones of the former are triterpenoids while those of the latter group are steroids analogous to the aglycones of digitalis glycosides. Holothurins in general appear to possess potent cardiotonic activity at low doses bordering on cardiotoxicity as observed on the isolated guinea pig heart (P. Kaul, B. Tursch, unpublished).

The well-known toxicity of starfishes is primarily due to the asterosaponins present in the starfish. These are lethal to fish (29–32) and toxic to annelids, molluscs, arthropods, and vertebrates (33, 34). Some of the purified saponins have been found, as would be expected, to cause hemolysis of fish and human erythrocytes blockable by cholesterol (31, 33). Asterosaponins from various marine organisms interfere in protein metabolism and possess cytolytic activity (35), anti-inflammatory, analgesic, and hypotensive actions (36), and neuromuscular junction blocking activity as observed in phrenic nerve–diaphragm preparations (37).

Like asterosaponins, holothurins from sea cucumbers are also ichthyotoxic (35, 38). The general toxicity of dilute holothurin solution has been demonstrated with coelenterates, nematodes, annelids, molluscs, crustaceans, frogs, and protozoa (39). The LD_{50} of holothurin-A in mice was found to be 9 mg/kg intravenously (i.v.) and 10 mg/kg i.p. (40). This compound elicits convulsions, rigidity of limbs, and respiratory depression (41). It is extremely toxic to mammals by the systemic route, but is nontoxic via the oral route because it is destroyed by stomach acid.

The holothurins possess potent hemolytic action blockable by cholesterol (42). Rats infected by *Trypanosoma lewisi* and subsequently treated with holothurin showed elevated parasitemia, but a reduced parasitemia was observed in rats given holothurin either prior to or simultaneously with the infection (43, 44). This partial protection against the parasite only when holothurin was given prior to the infection may suggest a possible interaction between the glycoside and the rat immune system.

The antifungal activity of various holotoxins (desulfated holothurins) can be ranked as follows: Holotoxin A = holotoxin B > holotoxin C > all the nine plant saponins tested (45). Holothurins are less active than the holotoxins. The polyene antibiotics, which are specifically antifungal, act by disrupting the membrane and rendering the fungal cell wall leaky (cholesterol can prevent the disruption). The antifungal activity of holotoxins and holothurins is probably also due to their ability to complex with or displace cholesterol. However, it is

questionable if this antifungal activity of the marine glycosides is specific, for these compounds also are potent cytolytic agents in general. A number of reports have appeared on the cytotoxic and antitumor activities of holothurins (35, 46–49).

Most of the neurotoxicity data on holothurins come from experiments on rat phrenic nerve–diaphragm and frog sciatic nerve–muscle preparations. Holthurin-A produced an irreversible block of both the neuromuscular junction and the muscle. However, both these responses were prevented by preincubating the preparations with dilute solutions (10^{-9} M) of anticholinesterases, e.g. physostigmine and neostigmine (37, 50–52). On the intact and internally perfused squid giant axon preparation, holothurin-A solution (10^{-4} M) increased Na^+ permeability, resulting in the nerve-membrane depolarization, apparently via the same general mechanism of action as the holothurins, i.e. complexation with the membrane cholesterol (53).

The effects of marine glycosides on Na^+-K^+ ATPase –dependent active transport have been studied more recently (54). Several triterpenoid glycosides, tested for their inhibitory activity on the rat brain Na^+-K^+ ATPase and Mg^{2+}-ATPase in vitro, showed a concentration-dependent inhibition of both the enzymes. The differences in the sugar moiety of the glycosides did not seem to matter, but the absence of sulfate groups appeared to enhance the Na^+-K^+ ATPase inhibition over that of the Mg^{2+}-ATPase. In general, the triterpenoid glycosides of sea cucumbers are more potent inhibitors of the ATPases than the steroidal glycosides from starfishes. All of the glycosides, however, lose their ATPase as well as other activities after pre-interaction with cholesterol (42, 54, 55).

In a bioassay-guided isolation of a CNS-depressant and hypothermic principle from *Holothuria floridana*, a saponin possessing potent and dose-dependent hypothermic and hemolytic activities was obtained that on hydrolysis yielded a known genin (16; P. Kaul and F. Schmitz, unpublished), griseogenin (Figure 1).

Peptides

ANTHOPLEURINS One of the pharmacologically most interesting groups of marine peptides is the group of anthopleurins isolated from *Anthopleura xanthogrammica* (Brandt) and *A. elegantissima* (Brandt), which yield anthopleurins A and B from the former (56) and C from the latter coelenterate (57). Anthopleurins (AP) are homologous peptides of 47–49 amino acid residues. On sequencing, the presence of 49 amino acids was revealed in AP-A, with three intramolecular disulfide bridges (58). These can be distinguished from AP-C by their two additional residues, and different residues at four locations (Figure 2).

AP-A produces a strong positive inotropic effect in various animal species without any effect on heart rate, blood pressure, or Na^+-K^+ ATPase. The

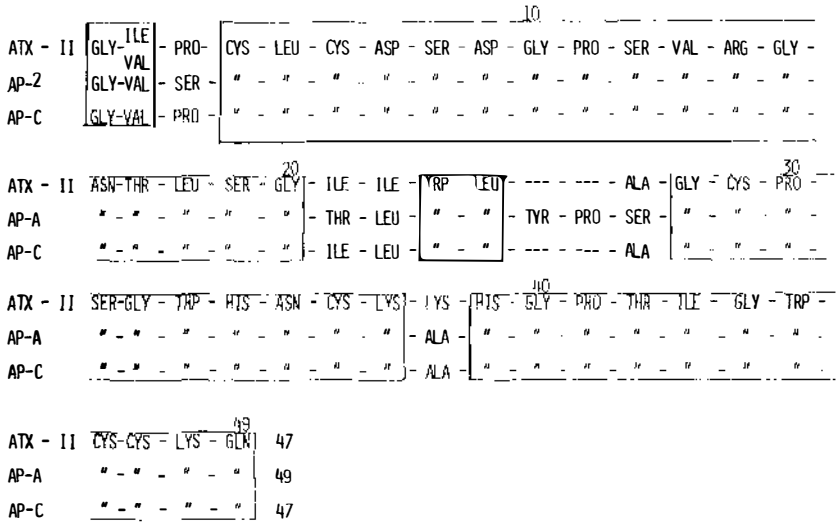

Figure 2 Amino acid sequences of anthopleurins A and C (AP-A, AP-C) compared to *Anemonia sulcata* toxin ATX-II. The boxed areas show common amino acids; the in-between areas show the differences in residues.

cardiotonic effect is unaffected by pretreatment with reserpine or α- and β-adrenergic blockers (59). In conscious dog, AP-A was found to be 35 times as cardiotonic as digoxin and nearly one third as toxic (60). Unlike digoxin, AP-A produced no ventricular extrasystole prior to the onset of ventricular fibrillation. At a single 2 μg/kg i.v. dose, AP-A increased the left ventricular pressure for over 2 hr in the conscious dog.

Conformational studies on AP-A with Laser-Raman and fluorescence spectroscopies reveal the molecule to be spherical with three pairs of CYS-CYS disulfide bridges (61). Correlation of structural modifications with cardiotonic activity revealed that the reduction of the disulfide bridges resulted in loss of activity and that most likely one or both of the lysine residues may be involved in mediating the response (62).

As a peptide of 5183 daltons, AP-A induces immune responses when given systemically to animals and loses activity when given orally (62). Obviously, systematic hydrolyses need to be carried out to explore the possibility of obtaining a smaller peptide that is either devoid of anaphylactic properties or is orally effective. This is analogous to the case of natural gastrin, which was replaced by an equally active pentapeptide, pentagastrin. The evidence that smaller peptides can be cardiotonic has come from another marine molecule, FMRFamide, a molluscan neurosecretory peptide. This tetrapeptide (Phe-Met-Arg-Phe-NH_2) stimulates cardiac as well as other excitable tissues (63). Further

studies are clearly warranted to see if a tetra- or a pentapeptide can be obtained from AP-A, which retains the cardiotonic activity of the parent molecule.

CARDIOTOXIN-II Biologically active polypeptides from sea anemones, resembling anthopleurins in both the amino acid sequence and cardiotonicity, have been studied extensively (64–70). Although seven peptides ranging from 2678 to 5630 daltons have been discovered (64), only anemonia toxin ATX-II has been studied at length.

A peptide of 47 amino acid residues (4770 daltons), ATX-II evoked a potent and dose-dependent cardiotonic response in various mammalian heart preparations. The changes in the steady-state cellular concentrations of Na^+, K^+, and Ca^{2+} affected by this peptide do not seem to account for its positive inotropic activity (65). Like AP-A, ATX-II also contains three pairs of cystein residues giving rise to three disulfide bridges and the resulting bifolded conformation (66). The primary difference between the amino acid sequences of ATX-II and AP-A lies in the charged center at residue 38, where second lysine of ATX-II is replaced by alanine in AP-A. This is 2–3 times as potent as the second lysine. This difference in the relative potencies has been attributed to this alanine/lysine variance, as well as to the considerably lower pKa value of the aspartate at residue 9 in ATX-II (69). Further structure-activity correlational studies have revealed that any modification of COOH in residue 9–aspartate or of arginine led to complete inactivation of ATX-II (70). It is conceivable that ionic dissociation, and thereby ionic and/or hydrogen bonding capability, of both these residues are involved in the interaction of ATX-II and the biological membrane receptors.

The cardiotonic effect of ATX-II is apparently not caused by a nonspecific membrane damage or by a direct action on the Na^+-K^+ ATPase, but by an indirect effect on the pump activity induced by increased Na^+ transport as a result of a delayed inactivation of fast sodium current (66). Thus, the sodium channels of the mammalian heart muscle cells appear to be the direct target for ATX-II. Further evidence in support of this sodium channel–specific action of this peptide has recently come from work on frog and mouse nerve–muscle preparations (68). The most characteristic action of ATX-II on the voltage-clamped denervated fast twitch muscle of the mouse is to slowly inactivate the Na^+ current. When this occurs, the rate constant of inactivation shows a reversed voltage dependence in the presence of the peptide. The significance of conformational disposition of these cardiotonic peptides from various sea anemones in relation to their activity was suggested originally for anthopleurins (57) but reemphasized recently for other peptides as well. The data obtained from comparative studies on AP-A and ATX-II, using circular dichroism and infrared spectroscopy and by application of modified Chou-Fasman method,

suggest a similarity of the overall conformation of these peptides (71). This, coupled to their largely similar amino acid sequences, basicities, and three disulfide bridges, could explain their almost identical pharmacological activities. Similarity of cardiotonic activity of ATX-II to that of AP-A was emphatically pointed out years ago (64; L. Beress, personal communication).

OTHER PEPTIDES Some of the less-studied peptides include a 147-residue peptide (20,000 daltons) from the sea anemone *Actinia equina,* which causes rapid hypotension, bradycardia, respiratory arrest in the intact rat, and pulmonary edema in preparations of the isolated rat lung (72); a 195-residue peptide (18,333 daltons) from the sea anemone *Condylactis gigantea,* which shows hemolytic action in rabbits blockable by sphingomyelin and is lethal to crayfish at nanogram doses (73); one of the smallest neurotoxic peptides (2000 daltons) from the sea anemone *Parasicyonis actinostoloides,* with an irreversible toxic action specific to crustacean neuronal tissue (74); several proteinase inhibitory, toxic, and hemolytic peptides ($\simeq$6,000 daltons) from sea anemones of *Stoichactis* sp. (75, 76); a vasoactive and cardiotonic peptide (12,000 daltons) from a coral, *Goniopora* sp., which prolongs action potential in amphibian and mammalian atria caused by delayed inactivation of Na^+ current (77), analogous to the mechanism of action of ATX-II (66); and cytolasin A-III from the marine heteronemertine *Cerebratulus lacteus,* a highly basic protein (10,000 daltons) capable of binding to membrane macromolecules, with the help of its C-terminal region in an amphipathic helical conformation, and consequently able to release liposomal markers (78–80).

Autonomium

An unusual compound from *Verongia fistularis* (81) with an isosteric hybrid structure of epinephrine and acetylcholine, autonomium (Figure 1) exhibited not only α- and β-adrenergic activities blockable by the respective antagonists, but it also possessed cholinergic activity typical of acetylcholine. The distance between the quaternary nitrogen and the oxygen-bearing carbon in autonomium is 4.2 Å as compared with 4.1 Å for the same distance in acetylcholine (16). This interfunctional group distance may perhaps explain the cholinergic activity in autonomium. Likewise, the adrenergic activity may be gauged from the autonomium's β-phenethylamine structure typical of epinehrine. It is not uncommon to find biologic activities in compounds with structure and interatomic distances between functional sites similar to those of known naturally occurring bioactive compounds.

Autonomium also shows CNS-stimulant effects in mice as gauged by a significant increase in spontaneous motor activity (81). The importance of such dual autonomically active compounds in marine environments is unknown, but it poses a question on the possibility of the occurrence of similar compounds in

mammalians. Such compounds within CNS could conceivably play a role in regulating behavior, since much has been written on the balance between the cholinergic and the adrenergic systems within CNS required for normal behavior of both animals and man.

CYTOTOXIC/ANTICANCER COMPOUNDS

Perhaps hundreds of thousands of extracts, fractions, and compounds of marine origin have been screened by the NCI national testing program during the past three decades. Although the NCI's in vitro (KB) and in vivo (PS) tests have found as much as 10.9% of the tested samples from marine organisms to be active (82), only a few compounds have reached some degree of prominence and none other than cytosine arabinoside (ara C) is used clinically.

Cembranoids

Several dozens of 14-membered cyclic diterpenes (cembranoids) have been isolated from various species of soft corals and chemically characterized (82–85). Sinularin and its dihydro congener, both diterpene cembranoids with exocyclic lactones obtained from the soft coral *Sinularia flexibilis,* were found to be effective in the NCI's screens of potential anticancer agents (82). Crassin acetate (Figure 1) from the Caribbean gorgonian *Pseudoplexaura porosa* was found to possess no significant pharmacological activity in various mammalian systems and preparations but was cytotoxic (1–10 μg/ml) to mouse fibroblasts and human leukemic and HeLa cells in vitro (86). However, when this cembranoid was dissolved in dilute alkaline solutions in which the lactone opens up, the cytotoxic activity against cancer cells was lost several hundredfold. This observation should be a stimulus to medicinal chemists involved in structural design of drugs and structure-activity relational studies.

Some of the cembranoids have been found to deciliate protozoa (87) and larvae of a nudibranch (85). Of the five compounds tested, peunicin (5 ppm) was the most potent, paralyzing the larvae within 10 min of exposure. It is conceivable that some of these cembrane lactones or their structural modifications may be parasiticidal or spermicidal. These compounds clearly warrant further pharmacological investigations, since mammals tolerate the compounds relatively well (86).

Depsipeptides

Didemnins A, B, and C, cyclic depsipeptides from the Caribbean tunicate, *Tridemnum* sp., possess novel structural features (88). These compounds are potent inhibitors of L1210 leukemic cells in vitro, are active against P-388 leukemia and B-16 melanoma in vivo, and protect mice infected intravaginally with *Herpes simplex* type-2 virus (89). Clinical trials and other tests have

revealed a high general toxicity of these compounds, but structural alterations and further testing should offer some hope in these novel compounds, particularly against *Herpes* infections.

Other Cytotoxic Agents

Antitumor and immunosuppressive effects of extracts and purified fractions of the sea squirt (tunicate) *Ecteinascidia turbinata* have been reported extensively since 1972, but to date no pure compound(s) with either of these activities has been isolated (90–93). An aqueous-alcoholic extract of the ascidian strongly inhibited the semiconservative DNA synthesis in human fibroblasts (93), which apparently is the primary mechanism of action for several antitumor compounds. However, in the ultraviolet-irradiated fibroblasts, only a weak inhibition of DNA excision repair was observed, which was also the case with arabinofuranosyl nucleosides, e.g. ara C and ara A (93). It should be interesting to witness the isolation of a pure molecular entity from *E. turbinata* possessing the claimed anticancer and immunomodulatory activities.

Of a series of 26-membered macrolides isolated from *Bugula neritina*, bryostatin and dolastatin have shown antileukemic activity in the NCI testing program (94). These macrolides are nearly twice the ring size of the anticancer cembranoids and represent a new class of potential anticancer molecules warranting further exploitation. A relatively new linear alcohol, triaconta-4,15,26-triene-1,12,18,29-tetrayne-3,14,17,28-tetraol, from a sponge of *Tetrosia* sp., has been found to inhibit mitosis in the sea urchin eggs at a concentration of 1 mg/ml (95). A number of similar polyacetylene alcohols have been isolated over the past several years, but their biological activity is yet to be determined.

An interesting observation has emerged more recently in the area of marine nucleosides, which had been found to occur only in sponges until a report appeared (96) that some of these nucleosides were isolated from the gorgonian *Eunicella cavolini*. Furthermore, the well-known antileukemic drug, ara A (9-B-D-arabinosyladenine), was originally developed as a semisynthetic modification of naturally occurring marine nucleosides, but now it has been found for the first time to exist in nature (96). Spongouridine, previously isolated from the sponge *Cryptotethia crypta* and found to possess antiviral activity, was also isolated from the coral *E. cavolini*.

A number of miscellaneous antitumor compounds and tumor promoters from marine prokaryotic organisms, especially the blue-green algae, have been described (97). A cyclic depsipeptide, majusculamide C from the alga *Lyngbya majuscula*, was found to partially inhibit X-5563 myeloma (0.5 mg/kg), but had no activity against P-388 leukemia, 6C3HED lymphoma, and 755 carcinoma in animal models. It is noteworthy that this compound resembles in structure the didemnins, which are also novel depsipeptides. However, didemnins have

exhibited more potent and clear anticancer potential, though accompanied by extreme toxicity. Other carcinogenic-carcinostatic macromolecules from blue-green algae include aplysiatoxin, lingbyatoxin, teleocidins A and B, and oscillatoxins. Obviously, most of these potent macromolecules are far too toxic to be of practical clinical value as such. However, semisynthetic manipulations and structure-activity relationships should yield a viable avenue to the discovery of new anticancer agents.

Based on a good correlation observed for known anticancer agents between their potency and their ability to inhibit (*a*) microtubule assembly in the microtubule polymerization assay utilizing purified bovine brain microtubule proteins, and (*b*) synchronous cell division of the fertilized sea urchin eggs, 130 known and structurally defined marine organic compounds were screened by the two assays for potential anticancer activity (98). Nine compounds were found to inhibit the first division of the sea urchin embryo. Of these, elatol from *Laurencia elata* and its oxidation product, elatone, were found quite effective in the cell division assay, but only elatone was effective in the microtubule polymerization assay. This reflects some selectivity and specificity inherent in the latter assay. Subsequently, a polycyclic orthoquinone, stypoldione, from the brown seaweed *Stypodium zonale* was found to be a potent inactivator of soluble tubulin and thereby an inhibitor of the microtubule polymerization (99).

OTHER PHARMACOLOGIC ACTIVITIES

Enzyme Inhibitors

While pursuing the bioassay-guided isolation of a CNS-depressant activity in the crude extracts of sea hare, *Alysia dactylomela,* an unsaturated and halogenated cyclic ether, dactylyne (Figure 1), was isolated (100). This compound produced a dose-dependent prolongation of pentobarbital-induced hypnosis in animals but by itself did not have any other apparent effects. Detailed studies on its mechanism of action revealed that it inhibits the metabolism of pentobarbital (101). Other related halogenated cyclic ethers with exocyclic enine features also showed this pentobarbital potentiation, but dactylyne was the most potent of the compounds tested (102).

Since pentobarbital is eliminated largely by oxidation involving Cyt-P_{450}, we had proposed that through inhibition of this enzyme dactylyne might prove to be a general drug metabolism inhibitor of clinical value (103). Subsequent work has revealed that dactylyne does indeed reversibly bind to Cyt-P_{450}, as determined by spectral changes of the enzyme, and that it consequently inhibits the activity of the enzyme (P. Kaul and I. Schuster, unpublished). Enzyme inhibitors in molecular biology are becoming increasingly important in controlling cellular processes. The marine-derived enzyme inhibitors should be screened for activity against other enzymes of clinical significance. Further-

more, structural modifications for rigorous structure-activity studies may yield desired activities with specificity, the ultimate aim in designing restriction enzymes or enzyme inhibitors.

Anti-inflammatory and Antispasmodic Agents

Flexibilide, a diterpenoid cembrane from the soft coral *Sinularia flexibilis*, dendalone 3-hydroxybutyrate from the sponge *Phyllospongia dendyi*, and 6-*n*-tridecylsalicylic acid from the brown alga *Caulocystis cephalornithos* were found to be orally effective anti-inflammatory agents at 20–200 μmol/kg doses; the dendalone compound is the most potent but also most toxic (104). These marine compounds provide a different structural concept for anti-inflammatory activity from the usually accepted types of such pharmacologic agents.

Manoalide, a nonsteroidal anti-inflammatory compound isolated from the sponge *Luffariella*
activity and a selective anti-inflammatory profile, suggesting that it acts by directly inactivating phospholipase A_2 (105). This enzyme is a component of several neurotoxins and is also involved in prostaglandin synthesis in man. Manoalide, when pre-incubated with β-bungarotoxin, prevented the irreversible neurotoxicity of the toxin on the rat phrenic nerve–diaphragm preparation (105).

Marine indole derivatives, other than Tyrian purple known for 80 years, have been discovered only within the recent decade and are not yet extensively subjected to pharmacologic studies. Around 100 indoles, including many alkaloids, have been isolated from marine plants, acorn worms, gorgonians, sea anemones, sponges, and bryozoans (106). The indole alkaloids flustramine A and B, isolated from Swedish marine organism *Flustra foliacae* L. of the phylum Bryozoa (moss animals), were reported as muscle relaxants both in vitro and in vivo (107). These compounds weakened the grip of mice in the screen grip test and inhibited the contractions of isolated but electrically stimulated rat diaphragm and guinea-pig ileum. Contractions evoked by histamine were also inhibited by the two flustramines. Their mode of action, though unknown, appears to be different from other known relaxants.

Ionophore Antibiotics

Being experimentally the easiest of most bioassays, the antibiotic assays and therefore the antibiotic activities of marine-derived extracts, fractions, and pure compounds have been reported with the largest frequency and numbers (5, 6, 10, 12, 105–9). Despite such reports during the past three decades, no antibiotic of clinical significance has emerged. This is perhaps understandable in view of the fact that it is very difficult to surpass several broad-spectrum and specifically potent antibiotics available.

Recently, however, a novel polyether antibiotic, acanthifolicin, was isolated

from the sponge *Pandaros acanthifolium* (110). In addition to being an interesting molecule structurally, it exhibits cytotoxic activity against certain cell lines but is lethal to mice at a 140 μg/kg i.v. dose. Another polyether cytotoxic compound, okadaic acid, has been isolated from two sponges of genus *Halichondria* (111). Also known as halichondrine-A, its LD_{50} in mice is 192 μg/kg i.v. It belongs to the group of compounds called ionophore polyethers capable of selectively carrying divalent ions such as Ca^{2+} across lipoidal membranes. However, in recent studies (112) okadaic acid caused contraction of various vascular smooth muscle preparations even in the absence of Ca^{2+}, a unique action indeed. This should reopen the question of mechanisms of smooth muscle contraction. Apparently, the role of okadaic acid–induced vascular contraction could not be explained by the Na^+K^+ pump involvement, since the acid has no effect on Na^+K^+ ATPase (112).

A group of unique boron-containing macrolide ionophores, aplasmomycins, has been isolated from the actinomycete *Streptomyces griseus,* probably of terrestrial origin, and found to be antimalarial against *Plasmodium berghei* (113). This may open inquiries into the development of new antimalarial drugs, as well as further exploration of marine microorganisms.

These types of polyether compounds have recently drawn much attention, but their toxicity appears to be relatively high. Whether or not any biomedically or clinically useful compounds will evolve out of these unique polyethers remains to be seen. There is no question, however, that these nearly linear macromolecules should be added to the list of other highly bioactive compounds from marine invertebrates that warrant investigation.

Latrunculins

Among scores of 14- and 16-membered macrocyclic diterpenes isolated from various corals and sponges, the most stimulating compounds perhaps are the latrunculins A (Figure 1) and B; these are isolated from a magnificent red-colored branching sponge, *Latruncula magnifica,* inhabiting the Red Sea at depths of 6–30 m (83, 114). The red juice expressed out of the fresh sponge into a fish aquarium agitates the fish within seconds and is lethal within 4–6 min apparently because of the latrunculin content of the juice.

Latrunculins at nanomolar concentrations induce profound changes in the microfilament organization without affecting the microtubules, an action strikingly similar to that of cytochalasins. In cultured mouse neuroblastoma clone NIE-115 and mouse fibroblasts, latrunculins (35 ng/ml) rapidly elicited morphological changes in the cells, which, however, were reversible upon removal of the marine compounds. Immunofluorescent studies revealed that the morphological changes resulted from the disruption of microfilaments at concentrations 1/10 to 1/100 that of cytochalasins. Also, unlike cytochalasins the latrunculins did not alter the rate of polymerization of the active filaments.

These differences have led to the belief that although the microfilament disruptive actions of the two classes of marine molecules are similar, their modes of action may be different (114).

Miscellaneous Activities

Aplysinopsin (Figure 1) from the yellow sponge *Verongia spengelii,* a tryptophan derivative, possesses cytotoxicity against KB, P-388, and L-1210 cell lines (115) as well as having an antidepressant profile in animals similar to that of imipramine (P. Kaul and S. Kulkarni, unpublished). When given orally, methylaplysinopsin from the sponge *Aplysinopsis reticulata* prevented the tetrabenazine-induced ptosis in mice and rats, inhibited MAO while simultaneously increasing brain serotonin concentration, reduced the neuronal reuptake of serotonin, and caused a generalized potentiation of serotonergic neurotransmission (116).

There are several dozens of compounds from marine invertebrates whose structures have been established, but on which only partial and preliminary pharmacological studies have been conducted. Some of these have shown hypotensive, antiarrhythmic, neuromuscular blocking, CNS depressant, and hypothermic activities (P. Kaul and F. Schmitz, unpublished).

MARINE TOXINS

It is beyond the scope of this review to include all of the marine toxins described as crude extracts, fractions, factors, amorphous mixtures, and structurally undefined molecules. Furthermore, it makes little sense to describe pharmacology of impure mixtures, because the pharmacology-toxicology and mode of action of pure toxins may eventually turn out to be quite different from parent crude materials. Therefore, this review is limited to only pure crystalline and structurally determined toxins, or those toxins whose homogeneity has been established prior to extensive studies. Even among these, only those toxins have been included that have either very potent activities or present an unusual mode of action.

Red-Tide Toxins

These toxins are produced by dinoflagellates that bloom periodically to produce both the toxins and the red carotenoid pigment, peridinin, believed to be responsible for the term "red tide." The main noxious species of this group of marine organisms include *Gonyaulax catenella, G. tamarensis,* and *Ptychodiscus brevis* (formerly known as *Gymnodinium breve*). The toxins accumulate in the shellfishes known to have a symbiotic relationship with the dinoflagellates; hence the name paralytic shellfish toxins.

The primary red-tide toxins isolated and studied at some length are saxitoxin (STX) produced by *G. catenella,* gonyautoxins (GTX_{1-5}) from *G. tamarensis,* and a number of lipid-soluble toxins from *P. brevis* (117). Like tetrodotoxin (TTX), the best-studied toxin, STX and GTXs are relatively polar heterocyclic molecules containing charged guanidinium groups.

SAXITOXIN AND GTXs Also known as paralytic shellfish poisons, these toxins exhibit a pharmacological-toxicological profile similar to that of TTX. Mechanistically, these guanidinium-containing molecules bind specifically to the sodium channels on the outside of excitable membranes, allowing an influx of Na^+ in exchange for K^+ eflux within a few milliseconds, with the attendant membrane depolarization. Various aspects relative to the specificity and stoichiometry of binding of STX and TTX to sodium channel receptors have been thoroughly established (118–121). Although TTX and STX have no direct effect on the K^+ channel receptors, a natural analogue of TTX, chiriquitoxin, does affect K^+ channels in addition to resembling TTX in the rest of its electrophysiological effects (121).

BREVETOXIN Within the past eight years, a large number of closely related toxins with varying and confusing nomenclature have been isolated from *Ptychodiscus brevis* and chemically characterized. This confusion has been somewhat cleared in a recent review (117), e.g. T_{34} and GB-2 toxins claimed to be different by their respective discoverers have now been established as the same compound on the basis of comparative physicochemical data. In general, the lipid-soluble toxins produced by *P. brevis* have been classified into either hemolytic or neurotoxic groups. It is not clear how many toxins are produced by *P. brevis* grown in laboratory cultures, but the overall preponderance appears to be of the neurotoxins rather than the hemolytic components. At least several neurotoxins have been isolated either as crystalline or homogeneous materials.

The structure of brevetoxin-B (BTX-B) reveals a cyclic polyether nature of the molecule, with 11 fused oxygen-containing rings, an α, β-unsaturated lactone, and an aldehyde function (122, 123). Thus, unlike the paralytic shellfish poisons (TTX, STX, and GTX), the BTX-B does not contain any nitrogen. Earlier work with crystalline and homogeneous brevetoxin(s) with no defined structure showed the toxin to cause centrally mediated cardiovascular and respiratory failures (124), neuromuscular blockade via increased Na^+ permeability (125), a TTX-blockable release of amino acids and acetylcholine from mammalian cortical synaptosomes (126), bronchoconstriction apparently mediated by ACh release (127), and a muscarinic crisis (128). Studies on the structurally defined BTX-B have revealed that it possesses positive inotropic and arrythmogenic activities on rat and guinea-pig hearts accompanied by A-V

block (129), ability to depolarize nerve membranes and terminals by activating the Na^+ channels at sites other than those specific for TTX and/or AP-A (130), and neuromuscular blocking activity resulting from persistent depolarization of the nerve terminal (131).

Specific polyclonal antibodies have been prepared against brevetoxins for trace detection of the toxins in the food chain (shellfish) prior to consumption, but attempts have failed to prevent the BTX-induced toxicological symptoms in fish by the use of these antibodies (132).

From the cyclic polyether structural information of at least one of the brevetoxins (BTX-B) it appears that there might be some similarities, in the yet to be uncovered biological activities, between the so-called ionophore polyethers and brevetoxins. For the present, these novel marine molecules are clearly bound to stimulate a lot of pharmacological and biochemical investigations.

Ciguatoxin

Although not directly related to the red tide, ciguatera poisoning in tropical and subtropical waters also results from periodic outbursts of dinoflagellates and is carried to man not through shellfish but through the consumption of some types of reef fishes. Ciguatera poisoning is characterized by neurological, gastrointestinal, and cardiovascular syndromes developing within 2 to 24 hours of eating the contaminated fish. The factor responsible for the toxicity was isolated from Pacific red snapper and termed ciguatoxin (CTX). It appears to be a complex lipid (133), and has recently been thought to originate from the dinoflagellate *Gambierdiscus toxicus* (134). Preliminary data have revealed the presence of one oxygen atom to every three carbons (117), suggesting that the CTX molecule is similar to the polyether toxins, e.g. BTX-B. Since pure crystalline CTX as a single molecular moiety was only recently obtained (135), its X-ray crystal structure should be forthcoming shortly, if it has not already been published before this review appears.

In anesthetized cats and rats, CTX at low doses (5–30 μg/kg, i.v.) elicited respiratory stimulation and bradycardia, while at higher but sublethal doses (40–80 μg/kg) respiratory depression as well as marked bradycardia and hypertension developed (136). At low concentrations in the perfusion fluid (100 pg/ml), CTX was found strongly cardiotonic (137), but this observation has been attributed to variabilities in tissue sensitivity and toxin purity (138). It also induces a marked release of norepinephrine (NE) from the presynaptic sites in the neuromuscular junction of guinea-pig vas deferens and causes supersensitivity at the postsynaptic sites due to an increase in the permeability of TTX-sensitive Na^+ channels in the smooth muscle membrane (139). However, the site of interaction for CTX is entirely different from that of any other site, making it a new type of Na^+ channel toxin among a total of six

different groups of toxins affecting Na^+ permeability, each by interacting with a specific site (140).

Palytoxin

One of the most interesting vasoactive molecules is palytoxin (PTX) isolated from various *Palythoa* species inhabiting the Caribbean (141) and Pacific (142) oceans. The structure of PTX has been elucidated (143) as a polyhydroxy, long chain macromolecule (Figure 3). It is the most potent marine toxin (144) and also the most potent coronary vasoconstrictor substance (16) known. As few as 1.6×10^{-17} moles can produce a nearly total constriction of the coronary artery in an isolated guinea-pig heart (145). Although this potent coronary vasoconstriction has been tentatively postulated as the mechanism of PTX toxicity (16), the toxin also has direct effects on a number of other muscle and nerve tissues (144–148).

The data on the action of PTX on nerve membranes indicate that the mechanism of action of PTX on the nerve tissues is clearly different from that of TTX. These data were obtained by intracellular and extracellular microelectrode recordings of neuronal activity in the coupled Retzius cells of horse leech ganglia. In these ganglia the membrane resistance as well as resting potential are unaffected by PTX (16). In both the nerve and the conducting system of the heart muscle, PTX appears to simulate the effects of large doses of extracellular

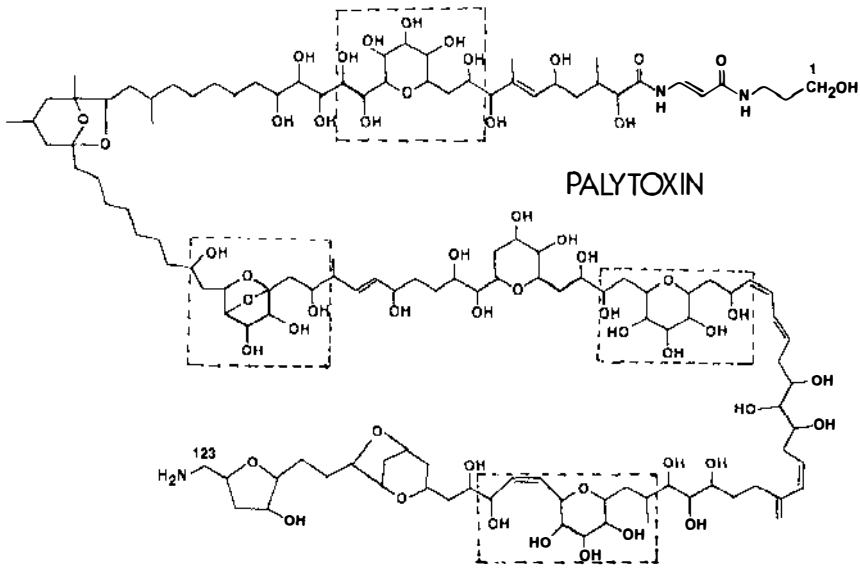

Figure 3 Structure of palytoxin (PTX). Boxed areas in dotted lines show possible chelation sites.

K^+ (16, 149, 150). In contrast to these observations, a decrease in the membrane resting potential in rabbit papillary muscle was noted in one study (151). However, it is questionable if this was really an effect of PTX, since a partially purified fraction of *Palythoa,* and not pure PTX, was used in this study. A biphasic contractile action of PTX observed on vas deferens has been attributed to a direct effect on the smooth muscle (first phase) and the release of NE from the adrenergic nerve terminals (second phase), since the second phase could be markedly inhibited by pretreatment with phentolamine or by predepletion of NE by reserpine (152).

An interesting effect of PTX is on the EKG of the rat, the dog, and the guinea pig, in which it elevates T-wave and S-T segment (16, 149) in a manner similar to that observed in patients suffering from variant or Prinzmetal angina (153). It is conceivable that PTX induces a coronary vascular spasm analogous to the spasm experienced in the variant angina. Thus, PTX may serve as a physiological tool to study animal models of this disease. Another very interesting observation is the potent inhibition of sperm motility induced by PTX (10^{-13} M) acting on the outer surface of the sperm membrane (154). Based on these studies, the use of sperm has been suggested for determining the mechanism of action of PTX.

The structure of PTX deserves an in-depth look, for it is one of the most unusual macromolecules in nature. There are several polyhydroxy and cyclic ether clusters (see dotted, boxed-in areas in its structural formula in Figure 3) suggestive of chelation sites. The molecule in general shows a high capability for both intra- and inter-molecular hydrogen bonding. It should also show a high binding capacity to biological macromolecules.

Other Marine Toxins

Halitoxin (HTX) from *Haliclona rubens,* the red "fire sponge", is a mixture of chemically defined heterocyclic molecules containing quaternary charged nitrogens as pyridinium moieties (155), analogous to curare alkaloids. It is a potent neuromuscular blocker and kills mice and rats by respiratory paralysis preceding cardiac arrest (P. Kaul, unpublished). Lophotoxin (LTX) from the sea whip coral (*Lophogorgia rigida*) is a cembranoid with two epoxides, an aldehyde, and an exocyclic lactone as its main chemical features (156). It has been claimed to be a new type of neuromuscular blocker acting on neuromuscular junction but on sites other than cholinoceptive receptors (157).

Maitotoxin (MTX) from the dinoflagellate *Gambierdiscus toxicus,* extensively studied though not yet chemically characterized, is a Ca^{2+} channel activator erroneously claimed (158) to be the most potent marine toxin known (See Table 1). It stimulates Ca^{2+} channels in insect skeletal muscles (159) and increases Ca^{2+} uptake in cultured NG108-15 neuroblastoma × glioma cells (160) by altering the voltage dependence of calcium channel activation.

Table 1 Relative toxicity of some of the marine toxins

Toxin	Organism	LD_{50}/kg (i.p.)	Species
Palytoxin	*Palythoa mammilosa*	50–100 ng	mice
Maitotoxin	*Gambierdiscus toxicus*	170 ng[a]	mice
Ciguatoxin	*G. toxicus*	450 ng	mice
Saxitoxin	*Saxidomus giganteus*	10 μg	mice
Tetrodotoxin	*Tapes semidecussata*	8–20 μg	mice
Laticatoxin	*Laticauda semifasciata*	130 μg	mice
Brevetoxin	*Ptychodiscus brevis*	250 μg	mice
Cephalotoxin	*Octopus vulgaris*	150–300 μg[b]	dogs
Lophotoxin	*Lophogorgia* sp.	8 mg	mice
Holotoxin	*Holothuria tubulosa*	5–15 mg[c]	mice
Nereistoxin	*Lumbriconeresis heteropoda*	33 mg[c]	mice
Halitoxin	*Haliclona viridis*	2.5 mg[c]	mice

[a]Minimum lethal dose.
[b]Subcutaneous.
[c]Intravenous.

Neosurugatoxin (NSTX) from the Japanese ivory mollusc, *Babylonia japonica,* is a potent blocker of sympathetic ganglia and a specific antagonist of nicotinic ACh receptors, as revealed by experiments on isolated guinea-pig ileum and radioligand binding studies with rat forebrain membranes (161). It is a nitrogenous heterocyclic compound with strong conjugation in a fused multiring system and contains sugar moieties.

BIOMEDICAL POTENTIAL AND BIOTECHNOLOGY

The biomedical potential of the sea has been emphasized repeatedly in recent decades (2, 6, 12, 14–17, 162). Clearly, a large number of unique organic molecules have been discovered and some of these have been found to possess either novel or potent pharmacologic activities. Of over 1000 structurally defined compounds of marine origin, only a score or two have been studied pharmacologically and only partially at that. This is so because in contrast to a relatively much larger number of dedicated marine natural-product chemists globally engaged in this field, only a handful of pharmacologists/biologists have been involved. Considering the usual ratio of one chemist to 4–6 biologists prevalent in industrial drug research, it is impossible to make serious progress in marine pharmacology/biomedicine unless many pharmacologists move toward the scientific novelties the sea has to offer. It is encouraging that ASPET in recent years has given some endorsement to marine pharmacology by including a few symposia on the subject, but, for a meaningful advance to occur in marine biomedicine, there will have to be a more substantial push from the

industry and from federal funding agencies to catalyze the involvement of more and competent chemists and pharmacologists.

Biotechnology, of course, has become the key word in every field, including electronics. It certainly has a realistic potential in marine pharmacology. It was quite an appropriate step, therefore, for the MIT Sea Grant Program to host a key lecture series on biotechnology interfacing with marine sciences (162). Application of genetic engineering to mariculture has already begun to demonstrate the potential of biotechnology in the marine sciences. It is a logical step to apply recombinant DNA technology to the production of reasonable amounts of otherwise oligopeptides and other potent bioactive molecules present in trace amounts in marine organisms. In fact, some investigators may already have begun in this direction. Approaches such as these will allow for new drug development at a rate commensurate with other advances both on Earth and in space.

CONCLUSION

The claims by skeptics that no new drugs have emerged from the sea despite the noise made by some of us fanatical marine pharmacologists and chemists must be viewed realistically. Firstly, not much effort is going on in the field compared to what has been and is now going into synthetic and terrestrial natural-products research—tens of thousands of chemists and biologists globally having spent hundreds of billions of dollars over the past 80 years. Secondly, at least several decades lapse between the first discovery of pharmacological activity of a compound and its final use as a drug. Thirdly, man has always gone to nature for a structural lead in just about every class of therapeutic agents. These facts, coupled with the evidence presented in this review of highly active and novel marine molecules, offer a challenge full of hope, which we can meet only by an aggressive and determined dip into the sea. We have no doubt whatsoever that the new drugs in the space-age of the twenty-first century will emerge from the oceans.

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