MARINE PHARMACOGNOSY

ACTION OF MARINE BIOTOXINS AT THE CELLULAR LEVEL

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Preface

The science of marine pharmacognosy—the study and utilization of marine drugs—is in many respects in a state of infancy. This statement must be tempered, however, with the acknowledgment that marine organisms and materials have been used from ancient times to cure or relieve various illnesses, ranging from stomachache to more exotic diseases. We also recognize that research in the field of marine pharmacognosy is accelerating and has attracted scientists trained in a range of disciplines, including agriculture, bacteriology, botany, chemistry, oceanography, pharmacology, physiology, and zoology. The marine bioactive substances being studied include a group that is among the most ubiquitious, the most potent, and the most diverse in its range of activity known to man. And yet its activity is, in many instances, poorly understood.

It appears timely, therefore, to bring together the present state of knowledge concerning the activity of selected marine drugs. The unifying theme of this volume—the use of marine biotoxins as probes of cellular functions—constitutes a useful means of understanding the information that has been obtained as well as the techniques and methods being used.

The contributions to this volume have three features. First, the contributors have presented details of procedures they have found useful. These include methods of isolation and characterization of bioactive agents, voltage-clamp techniques, kinetics of toxin-induced hemolysis, measurement of muscle contraction, toxin-induced alterations, bioassays, and microcalorimetry. Second, the contributions, individually and collectively, demonstrate the use and usefulness of marine bioactive agents as research tools. The uses may be one or more applications from a diverse range including removal of or binding to specific components of membranes, agents that

block specific physiological processes (cholinesterase inhibitors, general depolarizing agents, sodium pump blockers, etc.), antibiotics, and pesticides. Finally, the contributions help extend application of the techniques, procedures, and results to other relevant problems in physiology, pharmacology, and other fields. In a more general sense, this information will prove to be invaluable to scientists engaged in biological oceanography and comparative physiology.

We are grateful to Mrs. Susan Padilla for technical assistance.

Dean F. Martin
George M. Padilla

Contents

CHAPTER I

Marine Bioactive Agents: Chemical and Cellular Correlates

MARION T. DOIG, III, DEAN F. MARTIN, and GEORGE M. PADILLA

I. Introduction

A. Concept of Biodynamic Compounds

A natural compound from the sea usually comes to the attention of biologists because it exerts a striking (and most often toxic) effect on other organisms in the marine community. Greater emphasis has thus been

TABLE I

REPRESENTATIVE BIOACTIVE SUBSTANCES ISOLATED FROM MARINE ANIMALS

Taxonomic group	Genus and species	Compound(s)	Activity[a]	Reference
Porifera				
Sponges	*Microciona prolifera*	Ectyonin	Antibiotic	Nigrelli *et al.* (1959)
Coelenterata				
Hydroids	*Physalia physalis*	5-HT, low MW protein, and polypeptides	CNS, RS, NMS, ANS, CVS, GI	Lane *et al.* (1961)
Jellyfish	*Aurelia aurita*	—	CNS	Barnes and Horridge (1965)
Sea anemones	*Rhodactis howesii*	—	CNS, anticoagulant	E. J. Martin (1966)
Annelida				
Segmented worms	*Lumbriconereis heteropoda*	Nereistoxin	NMS, ANS, CVS, GI, anesthetic	Okaichi and Hashimoto (1962)
Mollusca				
Gastropods	*Haliotis* spp.	Paolin I Paolin II	Antibiotic Antiviral	C. P. Li *et al.* (1962, 1965)
Bivalves	*Mercenaria mercenaria*	Mercene	Antitumor	Schmeer (1966)
Octopus	*Octopus* spp.	Cephalotoxin, 5-HT, tyramine, octopamine	CNS, RS, NMS, ANS, CVS, GI, hemolytic	Hartman *et al.* (1960)
Arthropoda				
Lobsters	*Homarus americanus*	Homarine	CNS	Gasteiger *et al.* (1960)

Echinodermata				
Starfish	*Asterias* spp.	Saponins	CVS, hemolytic, sperm immobilization	Yasumoto *et al.* (1964)
Sea urchins	*Tripneustes gratilla*	Protein	CNS, RS, NMS, ANS, CVS	Alender *et al.* (1965)
Sea cucumbers	*Actinopyga agassize*	Holothurin A	NMS, hemolytic, antitumor	Nigrelli and Jakowska (1960)
Chordata				
Hagfish	*Eptatretus stoutii*	Eptatretin	CVS	Jensen (1963)
Sharks	*Hexanchus grisseus*	Ciguatera toxin	CNS, NMS, GI	der Marderosian (1968)
Stingrays	*Urobatis halleri*	Protein	CNS, RS, ANS, CVS, GI	Russell (1965)
Puffers	*Tetraodontidae*	Tetrodotoxin	CNS, RS, NMS, ANS, CVS, GI	Murtha (1960)
Mullet	*Mugil cephalus*	—	CNS, NMS, GI	der Marderosian (1968)
Weeverfish	*Trachinus vipera*	Protein venom, 5-HT	CNS, ANS, CVS	Russell and Emery (1960)
Scorpion fish	*Dendrochirus* spp.	Protein venom	CNS, RS, NMS, CVS	Russell (1965)
Newts	*Taricha torosa*	Tetrodotoxin	CNS, RS, NMS, ANS, CVS, GI	Mosher *et al.* (1964)
Turtles	*Chelonia mydas*	—	CNS, GI	der Marderosian (1968)
Snakes	*Pelamis platuras*	Protein	CNS, RS, NMS, ANS	der Marderosian (1968)

[a] Abbreviations: CNS, central nervous system; CVS, cardiovascular system; NMS, neuromuscular system; ANS, autonomic nervous system; RS, respiratory system; GI, gastrointestinal tract; 5-HT, 5-hydroxytryptamine.

placed on biotoxins or venoms than on any other class of compounds derived from the sea. This treatise is an attempt to compile studies on biotoxins. Yet the definition of compounds of interest to the scientific community must be widened beyond the area of toxicology to include whatever "bioactive compounds" may occur within natural products. This follows the suggestion of E.P. Chain (as cited by Halstead, 1968b) who used the similarly general term "biodynamic substance" to prompt the discovery of new drugs and toxins in what may be termed systematic toxinology. A bioactive substance is, therefore, any substance other than food that affects the structure and function of another organism in the marine environment. Biotoxins represent a somewhat more restrictive category, partly obscuring the fact that many "toxins" ultimately lead to the production of useful drugs, particularly after careful isolation, characterization, and alteration by synthetic means (Baslow, 1969, 1971). The term "drug" need not be limited to its strictly medical usage, but should be synonymous with the term "bioactive compound," as it also denotes any chemical substance that affects a specific physiological function (Fingl and Woodbury, 1965).

The main purpose of this chapter is to focus attention upon specific cellular effects of representative bioactive compounds and to correlate such pharmacological activities with the chemical uniqueness they possess. We have also sought to include other bioactive compounds not fully described in this volume in an attempt to acquaint the reader with new and, as yet, not fully investigated sources of pharmacological materials.

B. Pharmacology of Natural Products

A wide range of useful drugs has been isolated from plants (and animals). Although it is impossible to compile a full inventory, these drugs include analgesics, antibiotics, anticoagulants, antileukemic agents, cardioactive agents, enzymes, hormones, narcotics, and vitamins. The utility of these drugs was documented by a recent survey; more than 47% of new prescriptions contained drugs of natural origin as either the sole ingredient or as a component (der Marderosian, 1968).

The record of success in isolating new drugs from terrestrial plants is moderately impressive. At least 10,000 of the more than 400,000 species of plants have been screened chemically and/or pharmacologically to some degree. The successes are even more significant when we consider how many plant substances may have been less than thoroughly screened and prematurely rejected.

By comparison, the potential array of marine bioactive agents should be even more impressive. For example, approximately 80% of the earth's

TABLE II

REPRESENTATIVE BIOACTIVE SUBSTANCES ISOLATED FROM MARINE PLANTS

Taxonomic group	Genus and species	Compound	Activity[a]	Reference
Schizophyta (Bacteria)	*Flavobacterium piscicida*	—	CNS, antifugal, antiyeast	Halstead (1965)
Eumycophyta (Fungi)	*Cephalosporium acremonium*	Cephalothin	Antibiotic	Abraham (1962)
Cyanophyta (Blue-green algae)	*Nostoc rivulare*	—	Carcinogenic	Schwimmer and Schwimmer (1964)
Chlorophyta (Green algae)	*Chlamydomonas reinhardtii*	Fatty acids	Antibiotic	Starr (1962)
Chrysophyta (Golden algae)	*Prymnesium parvum*	Prymnesin	CNS, NMS, hemolytic, cytolytic, antispasmodic	Parnas (1963)
Pyrrophyta (Dinoflagellates)	*Gonyaulax catenella*	Saxitoxin	CNS, RS, NMS, ANS, CVS, GI	Bull and Pringle (1968)
Phaeophyta (Brown algae)	*Laminaria* spp.	Laminarin	Anticoagulant	Dewar (1956)
Rhodophyta (Red algae)	*Digenea simplex*	Kainic acid	Anthelmintic	Murakami *et al.* (1955)

[a] Abbreviations: CNS, central nervous system; CVS, cardiovascular system; NMS, neuromuscular system; ANS, autonomic nervous system; RS, respiratory system; GI, gastrointestinal tract.

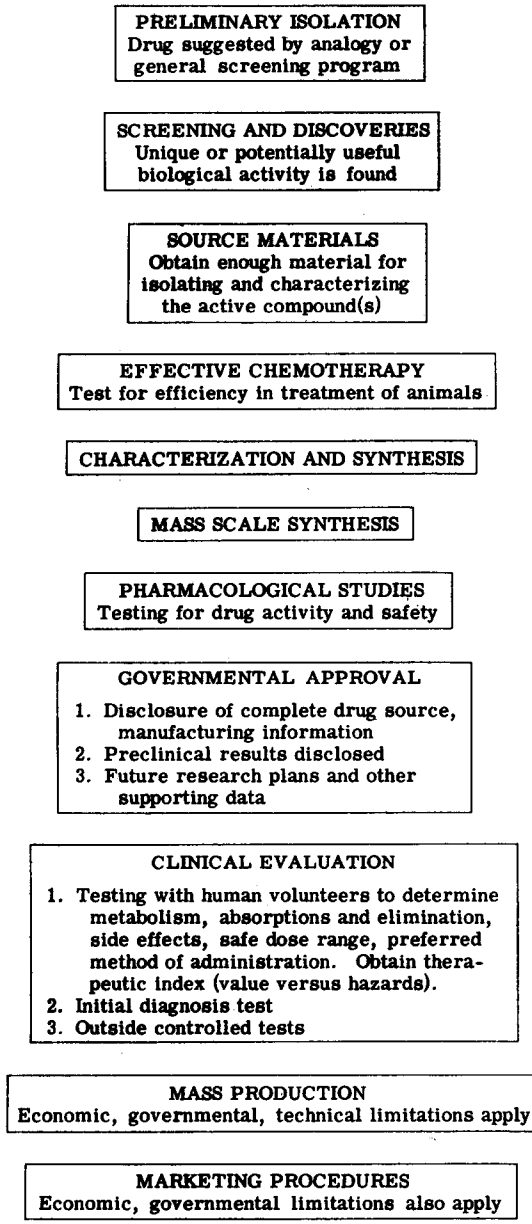

FIG. 1. Flow diagram for the isolation and production of drugs from marine sources.

animal life lives in the ocean and comprises 500,000 species in 30 phyla. And, according to Halstead (1965, 1967, 1968a), biotoxins are found throughout the entire phylogenetic series of marine animals (Table I). Of all these biotoxins, perhaps less than 1% have been examined for pharmacological activity. Probably, fewer than two dozen have been fully evaluated as to their chemical and pharmacological properties.

This paucity of known drugs from marine sources is surprising considering the potential value of plant sources alone (Table II). There are some explanations for this lack of substances. One problem is economic; it costs, on the average, about $40,000 to isolate an active agent and about $7,000,000 to develop and market from it a useful drug (D. F. Martin, 1970). A second problem is the lack of an interdisciplinary team (except in a few isolated situations) for isolating, screening, and developing the product. Other obstacles include those common to isolating drugs from terrestrial sources: harvesting and patent problems (Fig. 1).

Harvesting may be a formidable obstacle if an analogy to terrestrial organisms is valid. The useful drugs in these organisms (alkaloids, enzymes,

TABLE III

USEFUL BIOACTIVE COMPOUNDS ISOLATED FROM BENTHIC ALGAE

Compound	Source	Uses
Kainic acid (Fig. 4)	*Digenia simplex*	Anthelmintic against parasitic round worm (*Ascaris lumbricodes*), whip worm (*Trichuris trichura*), tapeworm (*Taenia* spp.)
Domoic acid (Fig. 6)	*Chondria armata*	Exterminates *Oxyics* and *Ascaris* worms
Laminine (Fig. 9)	*Laminaria augustata* (and about 20 species of Laminariaceae)	Hypotensive agent
Laminarin sulfate (Fig. 10)	*Laminaria caloustonii* and other spp.	Anticoagulant, heparinlike activity, antilipemic properties
Alginic acid (Fig. 11)	Kelp, brown seaweeds, *Fucus* spp., *Macrocystis* spp.	Tablet-disintegrating agents, derivative, blood anticoagulant sodium salt, inhibits strontium uptake from gastrointestinal tract
Carrageenan (Fig. 2)	*Chondrus crispus*	Antiviral activities, antiulcer properties, anticoagulant, antithrombic activity