# MARINE PHARMACOGNOSY

ACTION OF MARINE BIOTOXINS AT THE CELLULAR LEVEL



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ACTION OF MARINE BIOTOXINS AT THE CELLULAR LEVEL

#### EDITED BY

#### DEAN F. MARTIN

Department of Chemistry University of South Florida Tampa, Florida

### GEORGE M. PADILLA

Department of Physiology and Pharmacology Duke University Medical Center Durham, North Carolina



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ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by AGADEMIC PRESS, INC. (LONDON) LTD. 24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 72-84374

PRINTED IN THE UNITED STATES OF AMERICA

# List of Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

- M. A. Burklew, Florida Department of Natural Resources, Marine Research Laboratory, St. Petersburg, Florida (179)
- MARTIN T. Doig, III, Department of Chemistry, University of South Florida, Tampa, Florida (1)
- Angela F. Dulhunty, School of Physiology and Pharmacology, University of New South Wales, Kensington, Australia (85)
- Peter W. Gage, School of Physiology and Pharmacology, University of New South Wales, Kensington, Australia (85)
- MIYOSHI IKAWA, Department of Biochemistry, University of New Hampshire, Durham, New Hampshire (203)
- R. M. Ingle,\* Florida Department of Natural Resources, Marine Research Laboratory, St. Petersburg, Florida (179)
- WILLIAM R. KEM, Department of Pharmacology and Therapeutics, The J. Hillis Miller Health Center, University of Florida College of Medicine, Gainesville, Florida (37)
- DEAN F. MARTIN, Department of Chemistry, University of South Florida, Tampa, Florida (1, 265)
- Toshio Narahashi, Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina (107)
  - \* Present address: Conservation Consultants, Inc., Tallahassee, Florida.

- GEORGE M. Padilla, Department of Physiology, and Pharmacology, Duke University Medical Center, Durham, North Carolina (1, 265)
- ZVI PASTER, Department of Zoology, Tel-Aviv University, Tel-Aviv, Israel (241)
- JOHN J. SASNER, JR., Zoology Department and Jackson Estuarine Laboratory, University of New Hampshire, Durham, New Hampshire (127)
- K. A. Steidinger, Florida Department of Natural Resources, Marine Research Laboratory, St. Petersburg, Florida (179)
- RICHARD F. TAYLOR, Department of Biochemistry, University of New Hampshire, Durham, New Hampshire (203)

# 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

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#### 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
	B. Pharmacology of Natural Products
	C. Factors Affecting Distribution and Occurrence of Bioactive Agents
II.	Major Characteristics
	A. Physical and Chemical Properties.
	B. Physiological Properties
III.	Chemical and Cellular Correlates
	A. Saxitoxin-Tetrodotoxin
	B. Alginic Acid
IV.	Conclusions
	References

#### J. 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

FABLE I

REPRESENTATIVE BIOACTIVE SUBSTANCES ISOLATED FROM MARINE ANIMALS

Taxonomic group	Genus and species	Compound(s)	Activity <sup>a</sup>	Reference
Porifera				
Sponges	Microciona prolifera	Ectyonin	Antibiotic	Nigrelli <i>et al.</i> (1959)
Coelenterata				
$\mathbf{H}_{\mathbf{y}}$ droids	Physalia physalis	5-HT, low MW protein, and poly-	CNS, RS, NMS, ANS, CVS, GI	Lane et al. (1961)
		peptides		
Jellyfish	Aurelia aurita	1	CNS	Barnes and Horridge (1965)
Sea anemones	Rhodactis howesii	1	CNS, anticoagulant	E. J. Martin (1966)
Annelida				
Segmented	Lumbriconereis	Nereistoxin	NMS, ANS, CVS, GI,	Okaichi and Hashimoto (1962)
worms	heteropoda		anesthetic	
Mollusca				
Gastropods	Haliotis spp.	Paolin I	Antibiotic	C. P. Li et al. (1962, 1965)
		Paolin II	Antiviral	
Bivalves	Mercenaria mercenaria	Mercene	Antitumor	Schmeer (1966)
Octopus	Octopus spp.	Cephalotoxin, 5-HT, tyramine, octopamine	CNS, RS, NMS, ANS, CVS, GI, hemolytic	Hartman <i>et al.</i> (1960)
Arthropoda Lobsters	Homarus americanus	Homarine	CNS	Gasteiger et al. (1960)

Echinodermata Starfish	Asterias spp.	Saponins	CVS, hemolytic, sperm	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
Chordata Hagfish Sharks	Eptatretus stoutii Hexanchus grisseus	Eptatretin Ciguatera toxin	CVS CNS, NMS, GI	Jensen (1963) der Marderosian (1968)
Stingrays	Urobatis halleri	Protein	CNS, RS, ANS, CVS, GI	Russell (1965)
Puffers	Tetraodomiidae	Tetrodotoxin	CNS, RS, NMS, ANS, CVS,	Murtha (1960)
Mullet	Mugil cephalus	Protein venom, 5-HT Protein venom Tetrodotoxin	CNS, NMS, GI	der Marderosian (1968)
Weeverfish	Trachinus vipera		CNS, ANS, CVS	Russell and Emery (1960)
Scorpion fish	Dendrochirus spp.		CNS, RS, NMS, CVS	Russell (1965)
Newts	Taricha torosa		CNS, RS, NMS, ANS, CVS,	Mosher <i>et al.</i> (1964)
Turtles	Chelonia mydas	—	CNS, GI	der Marderosian (1968)
Snakes	Pelamis platuras	Protein	CNS, RS, NMS, ANS	der Marderosian (1968)

· 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

ABLE II

REPRESENTATIVE BIOACTIVE SUBSTANCES ISOLATED FROM MARINE PLANTS

Taxonomic group	Genus and species	Compound	${ m Activity}^a$	Reference
Schizophyta	Flavobacterium	l	CNS, antifugal, antiyeast	Halstead (1965)
la) ıyta	Pescusus Cephalosporium	Cephalothin	Antibiotic '	Abraham (1962)
ta een	acremonium Nostoc rivulare	ì	Carcinogenic	Schwimmer and Schwimmer (1964)
_	Chlamydomonas	Fatty acids	Antibiotic	Starr (1962)
Chrysophyta	reunaruu Prymnesium parvum	Prymnesin	CNS, NMS, hemolytic,	Parnas (1963)
· (2)	Gonyaulax catenella	Saxitoxin	CNS, RS, NMS, ANS, CVS, GI	Bull and Pringle (1968)
	Laminaria spp.	Laminarin	Anticoagulant	Dewar (1956)
	Digenea simplex	Kainic acid	Anthelmintic	Murakami et al. (1955)

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

PRELIMINARY ISOLATION Drug suggested by analogy or general screening program

SCREENING AND DISCOVERIES Unique or potentially useful biological activity is found

SOURCE MATERIALS
Obtain enough material for isolating and characterizing the active compound(s)

EFFECTIVE CHEMOTHERAPY
Test for efficiency in treatment of animals

CHARACTERIZATION AND SYNTHESIS

MASS SCALE SYNTHESIS

PHARMACOLOGICAL STUDIES
Testing for drug activity and safety

#### GOVERNMENTAL APPROVAL

- 1. Disclosure of complete drug source, manufacturing information
- 2. Preclinical results disclosed
- 3. Future research plans and other supporting data

#### **CLINICAL EVALUATION**

- Testing with human volunteers to determine metabolism, absorptions and elimination, side effects, safe dose range, preferred method of administration. Obtain therapeutic index (value versus hazards).
- 2. Initial diagnosis test
- 3. Outside controlled tests

MASS PRODUCTION
Economic, governmental, technical limitations apply

MARKETING PROCEDURES
Economic, governmental limitations also apply

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 anticoagu- lant sodium salt, inhibits strontium uptake from gastro- intestinal tract
Carrageenan (Fig. 2)	Chondrus crispus	Antiviral activities, antiulcer properties, anticoagulant, antithrombic activity