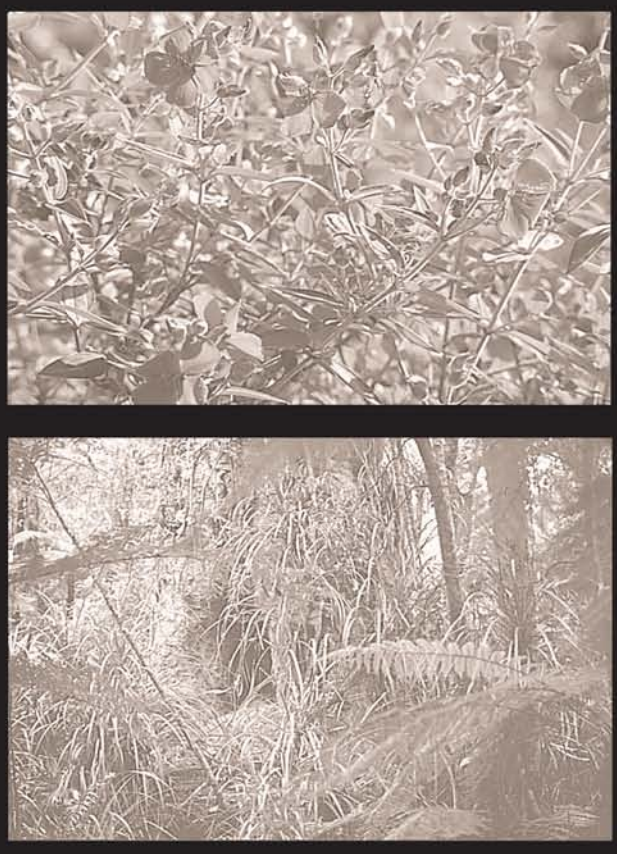
BIOLOGICALLY ACTIVE NATURAL PRODUCTS:
Pharmaceuticals



edited by
Stephen J. Cutler
Horace G. Cutler

BIOLOGICALLY ACTIVE NATURAL PRODUCTS:

edited by
Stephen J. Cutler
Horace G. Cutler

CRC Press
Boca Raton London New York Washington, D.C.

Contact Editor: Liz Covello
Project Editor: Maggie Mogck
Marketing Managers: Barbara Glunn, Jane Lewis
Arline Massey, Jane Stark
Cover design: Dawn Boyd

Library of Congress Cataloging-in-Publication Data

Biologically active natural products: pharmaceuticals / Stephen J. Cutler, Horace G. Cutler.
p. cm.
Includes bibliographical references and index.
ISBN 0-8493-1887-4 (alk. paper)
1. Pharmacognosy. 2. Natural products. I. Cutler, Horace G., 1932– . II. Title.
RS160.C88 1999
615'.321—dc21

99-13027
CIP

No claim to original U.S. Government works
International Standard Book Number 0-8493-1887-4
Library of Congress Card Number 99-13027
Printed in the United States of America 1 2 3 4 5 6 7 8 9 0
Printed on acid-free paper

Preface

Forty-five years ago, agricultural and pharmaceutical chemistry appeared to be following divergent paths. On the agricultural scene industrial companies were concentrating on the synthesis of various classes of compounds and when a successful chemical candidate was discovered, there was a good deal of joy among the synthetic chemists. We were told that as a result of chemistry life would be better and, indeed, it was. Armed with synthetic agrochemicals, the American farmer became the envy of the world. Essentially, with a vast series of chemical permutations, the synthetic chemist had tamed nature and the biblical admonition to subdue the natural world was well underway. One large agricultural chemical company, now out of the business, had in its arsenal plans to pursue "cyclohexene" chemistry among its many portfolios. Plans were already in motion to produce the series and on the drawing board was the synthesis of abscisic acid, later discovered in both cotton bolls and dormant buds of *Acer pseudoplatanus* as a biologically active natural product. The chemical elucidation led, in part, to the winning of the Nobel Prize by Dr. John W. Cornforth. How different the history might have been if the chemical company in question had synthesized the molecule quite by accident. In the field of pharmacy, natural product therapy was, at one time, the mainstay. With the rapid development of synthetic chemistry in the mid to late 1900s, those agents soon began to replace natural remedies. Even so, several natural products are still used today with examples that include morphine, codeine, lovastatin, penicillin, and digoxin, to name but a few. Incidentally, griseofulvin was first reported in 1939 as an antibiotic obtained from *Penicillium griseofulvum*. However, its use in the treatment of fungal infections in humans was not demonstrated until almost 1960. During the 20 years following its discovery, griseofulvin was used primarily as an agrochemical fungicide for a short period. Interestingly, it is a prescription systemic fungicide that is still used in medicine today.

Certainly, the thought that natural products would be successfully used in agriculture was a foreign concept at the beginning of the 1950s. True, the Japanese had been working assiduously on the isolation, identification, and practical use of gibberellic acid (GA) since the late 1920s. And later, in the early 1950s, both British and American plant scientists were busy isolating GA_3 and noting its remarkable effects on plant growth and development. But, during the same period, some of the major chemical companies had floated in and out of the GA picture in a rather muddled fashion, and more than one company dropped the project as being rather impractical. To date, 116 gibberellins have been isolated and characterized.

There was no doubt that ethylene, the natural product given off by maturing fruit, notably bananas (and, of course, smoking in the hold of banana ships was strictly forbidden because of the explosive properties of the gas) had potential, but how was one to use it in unenclosed systems? That, of itself, is an interesting story and involves Russian research on phosphate esters in 1945. Suffice it to say the problem was finally resolved on the practical level with the synthesis of the phosphate ester of 2-chloroethanol in the early 1970s. The chlorinated compound was environmentally benign and it is widely employed today as a ripening agent. Indole-3-acetic acid, another natural product which is ubiquitous in plants and controls growth and development, has been used as a chemical template, but has not found much use per se in agriculture. Indole-3-butyric acid, a purely synthetic compound, has large-scale use as a root stimulant for plant cuttings. The cytokinins, also natural product plant growth regulators, have found limited use since their discovery in stale fish

sperm, in 1950, mainly in tissue culture. Brassinolide, isolated from canola pollen, has taken almost 35 years to come to market in the form of 24-*epi*brassinolide and promises to be a highly utilitarian yield enhancer. However, there is no doubt that synthetic agrochemicals have taken the lion's share of the market.

In the 1980s something went wrong with the use of "hard" pesticides. Problems with contaminated groundwater surfaced. Methyl bromide, one of the most effective soil sterilants and all purpose fumigants, was found in well water in southwest Georgia. There was concern that the product caused sterility in male workers and, worse, the material was contributing to the ozone hole above the polar caps. Chlorinated hydrocarbons, such as DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane), were causing problems in the food chain and thin egg shells in wild birds was leading to declining avian populations. Never mind that following World War II, DDT was used at European checkpoints to delice and deflea refugees. The former ensured that the Black Plague, which is still with us in certain locations in the U.S., was scotched by killing the carrier, the flea. The elimination of yellow fever and malaria, endemic in Georgia in the early 1940s, also was one of the beneficial results of DDT. To date it is difficult to envisage that two thirds of the population of Savannah, GA was wiped out by yellow fever 2 years before the Civil War.

During the late 1980s and 1990s, a movement to use natural products in agriculture became more apparent. Insecticides, like the pyrethroids which are based on the natural product template pyrethrin, came to the marketplace. Furthermore, natural products had certain inherent desirable features. They tended to be target specific, had high specific activity, and, most important, they were biodegradable. The last point should be emphasized because while some biologically active organic natural products can be quite toxic, they are, nevertheless, very biodegradable. Another feature that became obvious was the unique structures of natural products. Even the most imaginative and technically capable synthetic chemist did not have the structural visions that these molecules possessed. Indeed, nature seems to make with great facility those compounds that the chemist makes, with great difficulty, if at all. This is especially true when it comes to fermentation products. It is almost a point of irony that agrochemistry is now at the same place, in terms of the development of new products, as that of pharmaceutical chemistry 50 years ago, as we shall see.

A major turning point in the pharmaceutical industry came with the isolation and discovery of penicillin by Drs. Howard W. Florey and Ernst B. Chain who, after being extracted from wartime England because of the threat of the Nazi invasion, found their way to the USDA laboratories in Peoria, IL, with the Agricultural Research Service. The latter, in those days, was preeminent in fermentation technology and, as luck would have it, two singular pieces of serendipity came together. First, "Moldy Mary", as she was called by her colleagues, had scared up a cantaloupe which happened to be wearing a green fur coat; in fact, *Penicillium chrysogenum*, a high producer of penicillin. Second, there was a byproduct of maize, corn steep liquor, which seemed to be a useless commodity. However, it caused *P. chrysogenum* to produce penicillin in large quantities, unlike those experiments in Oxford where Drs. Florey and Chain were able to produce only very small quantities of "the yellow liquid."

This discovery gave the pharmaceutical industry, after a great many delays and backroom maneuvering, a viable, marketable medicine. Furthermore, it gave a valuable natural product template with which synthetic chemists could practice their art without deleting the inherent biological properties. History records that many congeners followed, including penicillin G, N, S, O, and V, to name but a few. But, more importantly, the die was cast in terms of the search for natural product antibiotics and other compounds from fermentation and plants. That does not mean that synthetic programs for "irrational" medicinals

had stopped but, rather, that the realization that nature could yield novel templates to conquer various ills was a reality rather than a pipe dream. To use an old cliché, no stone would go unturned; no traveler would return home from an overseas trip without some soil sticking to the soles of his shoes.

The common denominator in both agrochemical and pharmaceutical pursuits is, obviously, chemistry. Because of the sheer numbers of natural products that have been discovered, and their synthetic offspring, it was inevitable that the two disciplines would eventually meld. Examples began to emerge wherein certain agrochemicals either had medicinal properties, or vice versa. The chlorinated hydrocarbons which are synthetic agrochemicals evolved into useful lipid reducing compounds. Other compounds, such as the benzodiazepine, cyclopenol from the fungus *P. cyclopium*, were active against *Phytophthora infestans*, the causal organism of potato late blight that brought Irish immigrants in droves to the New World in search of freedom, the pursuit of happiness, and, as history records, the presidency of the U.S. for their future sons; and, one hopes in the future, their daughters. While not commercially developed as a fungicide, the cyclopenol chemical template has certain obvious other uses for the pharmacist. And, conversely, it is possible that certain synthesized medicinal benzodiazepines, experimental or otherwise, have antifungal properties yet to be determined. It also is of interest to note that the β-lactone antibiotic 1233A/F, [244/L; 659, 699], which is a 3 hydroxy-3-methyl glutaryl CoA reductase inhibitor, has herbicidal activity. Interweaving examples of agrochemicals that possess medicinal characteristics and, conversely, medicinals that have agrochemical properties occur with increasing regularity.

In producing a book, there are a number of elements involved, each very much dependent on the other. If one of the elements is missing, the project is doomed to failure.

First, we sincerely thank the authors who burned the midnight oil toiling over their research and book chapters. Writing book chapters is seldom an easy task, however much one is in love with the discipline, and one often has the mental feeling of the action of hydrochloric acid on zinc until the job is completed. We thank, too, those reviewers whose job is generally a thankless one at best.

Second, we thank the Agrochemical Division of the American Chemical Society for their encouragement and financial support, and especially for the symposium held at the 214th American Chemical Society National Meeting, Las Vegas, NV, 1997, that was constructed under their aegis. As a result, two books evolved: *Biologically Active Natural Products: Agrochemicals* and *Biologically Active Natural Products: Pharmaceuticals*.

Third, the School of Pharmacy at Mercer University has been most generous with infrastructural support. The Dean, Dr. Hewitt Matthews, and Department Chair, Dr. Fred Farris, have supported the project from inception. We also thank Vivienne Oder for her editorial assistance.

Finally, we owe a debt of gratitude to the editors of CRC Press LLC who patiently guided us through the reefs and shoals of publication.

Stephen J. Cutler
Horace G. Cutler

Editors

Stephen J. Cutler, Ph.D., has spent much of his life in a laboratory being introduced to this environment at an early age by his father, "Hank" Cutler. His formal education was at the University of Georgia where he earned a B.S. in chemistry while working for Richard K. Hill and George F. Majetich. He furthered his education by taking a Ph.D. in organic medicinal chemistry under the direction of Dr. C. DeWitt Blanton, Jr. at the University of Georgia College of Pharmacy in 1989. His area of research included the synthesis of potential drugs based on biologically active natural products such as flavones, benzodiazepines, and aryl acetic acids. After graduate school, he spent 2 years as a postdoctoral fellow using microorganisms to induce metabolic changes in agents which were both naturally occurring as well as those he synthesized.

The latter brought his research experience full circle. That is, he was able to use his formal educational training to work in an area of natural products chemistry to which he had been introduced at an earlier age. He now had the tools to work closely with his father in the development of natural products as potential pharmaceuticals and/or agrochemicals either through fermentation, semisynthesis, or total synthesis. From 1991 to 1993, the younger Cutler served as an Assistant Professor of Medicinal Chemistry and Biochemistry at Ohio Northern University College of Pharmacy and, in 1993, accepted a position as an Assistant Professor at Mercer University School of Pharmacy. He teaches undergraduate and graduate pharmacy courses on the Medicinal Chemistry and Pharmacology of pharmaceutical agents.

Horace G. (Hank) Cutler, Ph.D., began research in agricultural chemicals in February 1954, during the era of, "we can synthesize anything you need," and reasonable applications of pesticides were 75 to 150 lb/acre. His first job, a Union Carbide Fellowship at the Boyce Thompson Institute for Plant Research (BTI), encompassed herbicides, defoliants, and plant growth regulators (PGRs); greenhouse evaluations, field trials, formulations; and basic research. He quickly found PGRs enticing and fell madly in love with them because of their properties. That is, they were, for the most part, natural products and had characteristic features (high specific activity, biodegradable, and target specific). After over 5 years at BTI, he went to Trinidad, West Indies, to research natural PGRs in the sugarcane, a monoculture.

It quickly became evident that monocultures used inordinate quantities of pesticides and, subsequently, he returned to the U.S. after 3 years to enter the University of Maryland. There, he took his degrees in isolating and identifying natural products in nematodes (along with classical nematology, plant pathology, and biochemistry). Following that, he worked for the USDA, Agricultural Research Service (ARS) for almost 30 years, retired, and then was appointed Senior Research Professor and Director of the Natural Products Discovery Group, Southern School of Pharmacy, Mercer University, Atlanta. He has published over 200 papers and received patents on the discovery and application of natural products as agrochemicals (the gory details are available at ACS online). Hank's purloined, modified motto is: "Better ecological living through natural product chemistry!"

Contributors

Maged Abdel-Kader Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Arba Ager Department of Microbiology and Immunology, School of Medicine, University of Miami, Miami, Florida

Feras Q. Alali Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana

Francis Ali-Osman Section of Molecular Therapeutics, Department of Experimental Pediatrics, M. D. Anderson Cancer Center, University of Texas, Houston, Texas

Khisal A. Alvi Thetagen, Bothell, Washington

Mitchell A. Avery National Center for the Development of Natural Products, Department of Medicinal Chemistry, School of Pharmacy and Department of Chemistry, University of Mississippi, University, Mississippi

Piotr Bartyzel National Center for the Development of Natural Products and the Department of Pharmacognosy, University of Mississippi, University, Mississippi

J. Warren Beach Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, Georgia

John M. Berger Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

John K. Buolamwini National Center for the Development of Natural Products, Research Institute of Pharmaceutical Sciences and Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, Mississippi

N. Dwight Camper Department of Plant Pathology and Physiology, Clemson University, Clemson, South Carolina

Leng Chee Chang Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois

Ha Sook Chung Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois

Alice M. Clark National Center for the Development of Natural Products, Research Institute of Pharmaceutical Sciences and Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi

Baoliang Cui Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois

William Day National Center for the Development of Natural Products and Department of Pharmacognosy, University of Mississippi, University, Mississippi

D. Chuck Dunbar National Center for the Development of Natural Products and Department of Pharmacognosy, University of Mississippi, University, Mississippi

Geoff Edwards Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, England

Khalid A. El Sayed National Center for the Development of Natural Products and Department of Pharmacognosy, University of Mississippi, University, Mississippi

Randall Evans Missouri Botanical Garden, St. Louis, Missouri

Lisa Famolare Conservation International, Washington, D.C.

Roy E. Gereau Missouri Botanical Garden, St. Louis, Missouri

Marianne Guerin-McManus Conservation International, Washington, D.C.

Mark T. Hamann National Center for the Development of Natural Products and Department of Pharmacognosy, University of Mississippi, University, Mississippi

Richard A. Hudson Department of Medicinal and Biological Chemistry, College of Pharmacy, and Departments of Biology and Chemistry, University of Toledo, Toledo, Ohio

Aiko Ito Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois

Holly A. Johnson Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana

A. Douglas Kinghorn Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois

David G. I. Kingston Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Kuo-Hsiung Lee Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina

Lina Long Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois

George Majetich Department of Chemistry, University of Georgia, Athens, Georgia

Stanley Malone Stichting Conservation, International Suriname, Paramaribo, Suriname

James D. McChesney NaPro BioTherapeutics, Inc., Boulder, Colorado

Christopher R. McCurdy Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, Georgia

Jerry L. McLaughlin Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana

Graham McLean Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, England

Sanjay R. Menon Department of Medicinal Chemistry and Department of Microbiology/Genetics, Kansas University, Lawrence, Kansas

James S. Miller Missouri Botanical Garden, St. Louis, Missouri

Reagan L. Miller Department of Pharmaceutical Sciences, College of Pharmacy, Ohio Northern University, Ada, Ohio

Lester A. Mitscher Department of Medicinal Chemistry and Department of Microbiology/Genetics, Kansas University, Lawrence, Kansas

Russell Mittermeier Conservation International, Washington, D.C.

Nicholas H. Oberlies Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana

Christine A. Pillai Department of Medicinal Chemistry and Department of Microbiology/Genetics, Kansas University, Lawrence, Kansas

Segaran P. Pillai Department of Medicinal Chemistry and Department of Microbiology/Genetics, Kansas University, Lawrence, Kansas

Eun-Kyoung Seo Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois

Delbert M. Shankel Department of Medicinal Chemistry, Department of Microbiology/Genetics, Kansas University, Lawrence, Kansas

Albert T. Sneden Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia

Fabio Soldati Research and Development, Pharmaton SA, Lugano, Switzerland

L. M. Viranga Tillekeratne Department of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, Toledo, Ohio

Hendrik van der Werff Missouri Botanical Garden, St. Louis, Missouri

Larry A. Walker National Center for the Development of Natural Products, Research Institute of Pharmaceutical Sciences and Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi

David E. Wedge USDA, Agricultural Research Service, The Center for the Development of Natural Products, School of Pharmacy, University of Mississippi, University, Mississippi

Jan H. Wisse BGVS Suriname, Geyersulijt, Suriname

Shu-Wei Yang Phytera, Inc., Worcester, Massachusetts

Bing-Nan Zhou Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Jordan K. Zjawiony National Center for the Development of Natural Products and Department of Pharmacognosy, University of Mississippi, University, Mississippi

Contents

1. Connections between Agrochemicals and Pharmaceuticals
   David E. Wedge and N. Dwight Camper

2. Fractionation of Plants to Discover Substances to Combat Cancer
   A. Douglas Kinghorn, Baoliang Cui, Aiko Ito, Ha Sook Chung, Eun-Kyoung Seo, Lina Long, and Leng Chee Chang

3. Therapeutic Potential of Plant-Derived Compounds: Realizing the Potential
   James S. Miller and Roy E. Gereau

4. Biodiversity Conservation, Economic Development, and Drug Discovery in Suriname
   David G. I. Kingston, Maged Abdel-Kader, Bing-Nan Zhou, Shu-Wei Yang, John M. Berger, Hendrik van der Werff, Randall Evans, Russell Mittermeier, Stanley Malone, Lisa Famolare, Marianne Guerin-McManus, Jan H. Wisse, and James S. Miller

5. Recent Trends in the Use of Natural Products and Their Derivatives as Potential Pharmaceutical Agents
   George Majetich

6. Highlights of Research on Plant-Derived Natural Products and Their Analogs with Antitumor, Anti-HIV, and Antifungal Activity
   Kuo-Hsiung Lee

7. Discovery of Antifungal Agents from Natural Sources: Virulence Factor Targets

   Alice M. Clark and Larry A. Walker

8. Reactive Quinones: From Chemical Defense Mechanisms in Plants to Drug Design
   Richard A. Hudson and L. M. Viranga Tillekeratne

9. Structure–Activity Relationships of Peroxide-Based Artemisinin Antimalarials
   Mitchell A. Avery, Graham McLean, Geoff Edwards, and Arba Ager

10. Naturally Occurring Antimutagenic and Cytoprotective Agents
    Lester A. Mitscher, Segaran P. Pillai, Sanjay R. Menon, Christine A. Pillai, and Delbert M. Shankel

11. Lobeline: A Natural Product with High Affinity for Neuronal Nicotinic Receptors and a Vast Potential for Use in Neurological Disorders
Christopher R. McCurdy, Reagan L. Miller, and J. Warren Beach

12. Protein Kinase C Inhibitory Phenylpropanoid Glycosides from *Polygonum* Species
Albert T. Sneden

13. Thwarting Resistance: Annonaceous Acetogenins as New Pesticidal and Antitumor Agents
Holly A. Johnson, Nicholas H. Oberlies, Feras Q. Alali, and Jerry L. McLaughlin

14. A Strategy for Rapid Identification of Novel Therapeutic Leads from Natural Products
Khisal A. Alvi

15. Dynamic Docking Study of the Binding of 1-Chloro-2,4-Dinitrobenzene in the Putative Electrophile Binding Site of Naturally Occurring Human Glutathione *S*-Transferase pi Allelo-Polymorphic Proteins
John K. Buolamwini and Francis Ali-Osman

16. *Panax ginseng:* Standardization and Biological Activity
Fabio Soldati

17. Marine Natural Products as Leads to Develop New Drugs and Insecticides
Khalid A. El Sayed, D. Chuck Dunbar, Piotr Bartyzel, Jordan K. Zjawiony, William Day, and Mark T. Hamann

18. Commercialization of Plant-Derived Natural Products as Pharmaceuticals: A View from the Trenches
James D. McChesney

1

Connections between Agrochemicals and Pharmaceuticals

David E. Wedge and N. Dwight Camper

CONTENTS

1.1 Introduction
1.2 General Characteristics
1.3 Pharmacokinetics
1.4 Pharmacodynamics
1.5 Direct-Acting Defense Chemicals — Mitotic Inhibitors and DNA Protectants
1.6 Indirect-Acting Defense Chemicals — Fatty Acid Inhibitors and Signal Transduction
1.7 New Chemistries and Modes of Action
1.8 Conclusions
References

ABSTRACT Antibiotics, antineoplastics, herbicides, and insecticides often originate from plant and microbial defense mechanisms. Secondary metabolites, once considered unimportant products, are now thought to mediate plant defense mechanisms by providing chemical barriers against animal and microbial predators. This chemical warfare between plants and their pathogens consistently provides new natural product leads. Whether one studies toxins, herbicides, or pharmaceuticals, chemical compounds follow basic rules of pharmacokinetics and pharmacodynamics. Chemical properties of a molecule dictate its cellular and physiological responses, and organisms will act to modulate those chemical responses. Discovery and development of new biologically derived and environmentally friendly chemicals are being aggressively pursued by leading chemical and pharmaceutical companies. Future successful development and approval of these new chemicals will require knowledge of their common mechanisms in toxicology and pharmacology regardless of their applications to plants or animals.

1.1 Introduction

Animals, including humans, and most microorganisms depend directly or indirectly on plants as a source of food. It is reasonable to assume that through evolution plants have

developed defense strategies against herbivorous animals and pathogens. Plants also must compete with other plants, often of the same species, for sunlight, water, and nutrients. Likewise, animals have developed defensive strategies against microbes and predators.[1-3] Examples include the complex immune system with its cellular and humoral components[4] to protect against microbes; weapons, armor, thanatosis (death), deimatic behavior, aposematism (conspicuous warning), flight; or development of a poison or defense chemical.[1] However, plants cannot move to avoid danger; therefore, they have developed other mechanisms of defense: the ability to regrow damaged or eaten parts (leaves); mechanical protection (i.e., thorns, spikes, stinging hairs, etc.); thick bark in roots and stems, or the presence of hydrophobic cuticular layers; latex or resins which deter chewing insects; indigestible cell walls; and the production of secondary plant metabolites.[5] The latter may be the most important strategy for plant defense. Examples of an analogous mechanism are found in many insects and other invertebrates, i.e., many marine species;[6] some vertebrates also produce and store protective metabolites which are similar in structure to plant metabolites. In some cases animals have obtained these toxins from plants, e.g., the monarch butterfly (*Danaus plexippus*) and the poison dart frogs (*Dendrobatidae*) found in the rain forests of Central and South America.[3,7,8] While the function of many plant secondary metabolites is not known, we can assume these chemicals are important for survival and fitness of a plant, i.e., protection against microorganisms, herbivores, or against competing plants (allelopathic interactions) and to aid in reproduction (insect attractants). Plants produce numerous chemicals for defense and communication, but also plants can elicit their own form of offensive chemical warfare targeting cell proliferation of pathogens. These chemicals may have general or specific activity against key target sites in bacteria, fungi, viruses, or neoplastic diseases.

Throughout recorded time humans have knowingly and unknowingly utilized plant metabolites as sources of agrochemicals and pharmaceuticals. Discovery of vincristine and vinblastine[9] in 1963 by R. L. Noble and his Canadian co-workers[10] and its successful usepatent by Eli Lilly launched the pharmaceutical industry into the search for natural product leads for the treatment of various cancers. Recent natural product discovery and development of avermectin (anthelmintic), cyclosporin and FK-506 (immunosuppressive), mevinolin and compactin (cholesterol-lowering), and Taxol and camptothecin (anticancer) have revolutionized therapeutic areas in medicine.[11] Similar successful development of azoxystrobin (β-methoxyacrylate) fungicides and spinosad (tetracyclic macrolides) pesticides have created a renewed interest in natural product agrochemical discovery. Because biologically derived chemicals are perceived by consumers as having less environmental toxicity and lower mammalian toxicity, chemical companies currently have a greater desire to discover and develop natural product-based plant protectants.

1.2 General Characteristics

Biological activity of a natural product involves several key characteristics that apply regardless of whether the activity is for an agrochemical or pharmaceutical application. One involves the classical dose–response relationship. Paracelsus recognized, in 1541, the need for proper experimentation to determine the toxic level of a chemical. He distinguished between therapeutic and toxic properties of a chemical and recognized that these may be indistinguishable except by dose. He stated: "All substances (chemicals) are poisons; there is none which is not a poison. The only difference between a remedy and a poison is its dose." Most drugs are therapeutic over a narrow range of doses; they are also toxic at higher doses. The problems of proper dose in order to achieve the desired pharmaceutically effective

concentration and other physiological parameters are the subject of pharmacokinetics. Activity of plant growth regulators is governed by the same principle. 2,4-Dichlorophenoxyacetic acid (2,4-D) is an effective herbicide at high concentrations (kg/ha), but at low concentrations (mg/l) it has growth promotive effects in *in vitro* plant culture systems. Relatively low concentrations of indole-3-acetic acid that promotes growth of stems (10^{-5} to 10^{-3} *M*) is inhibitory to roots as compared to that which promotes root growth (10^{-11} to 10^{-10} *M*).[12]

1.3 Pharmacokinetics

Agrochemicals and pharmaceuticals are subjected to absorption, metabolism, distribution, and excretion or compartmentalization. Metabolic action can result in activation or inactivation of the chemical. A fermentation product of *Streptomyces hygroscopicus*, Bialophos, is apparently converted to glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid]. Bialophos is used as a herbicide in field-grown and containerized nursery plants and is a glutamine synthase inhibitor.[13] In mammals, carbon tetrachloride is converted to an active toxic metabolite by cytochrome P450 and is active in inducing liver necrosis through a free radical mechanism.[14] Many other examples of metabolic changes could be cited which result in biotransformation to toxic or carcinogenic compounds. The converse of metabolic conversion is detoxification or degradation rendering the final product of the agrochemical or pharmaceutical inactive. Acetaminophen, transformed by N-oxidation to *N*-acetyl-*p*-benzoquinonimine, is rapidly conjugated to glutathione by glutathione transferase and channeled into the excretory system. The herbicide atrazine is detoxified by conjugation with glutathione by glutathione transferase in maize.[15]

Absorption, distribution, and excretion or compartmentalization influence the biological activity of both pharmaceuticals and agrochemicals. For pharmaceuticals, interactions of a chemical with an organism involves exposure, toxicokinetics, and toxicodynamics. Exposure is usually intentional and the chemical then undergoes absorption into the circulatory system, distribution among tissues, and elimination from the body or deposition in specific tissues. The extent to which an agrochemical is absorbed by plants depends on the anatomy of leaves, stems, and roots and the structural and chemical characteristics of the cuticle and epidermis. Membranes pose barriers to the absorption and distribution of both pharmaceuticals and agrochemicals. These cellular structures must be penetrated in order for the chemical to reach its site of action. Compounds that can easily cross cell membranes, through simple diffusion or active transport mechanisms, will be more easily absorbed than compounds which cannot. Chemicals with a high degree of lipid solubility may penetrate the cellular membrane more efficiently than a chemical which is more polar in nature. While excretion is not a usual method of distribution of an agrochemical from a plant, root exudation may occur for certain chemicals. Other chemicals may be sequestered in the plant vacuoles, or conjugated; both processes usually render the chemical biologically inactive or unavailable for induction of a physiological response.

1.4 Pharmacodynamics

Structure–activity relationships relate the chemical structure of a molecule to its affinity for a receptor and intrinsic or biological activity. Relatively minor modifications in the chemical

molecule may result in major changes in biological properties. This relationship was first proposed by Paul Ehrlich from his chemotherapy studies of arsenicals effective against syphilis in the late 1800s. He also postulated the existence of receptors in trying to explain the stereospecificity of drug effects. Changes in molecular structure affect physical/chemical properties of the molecule such as solubility, hydrophilic/hydrophobic balance (partition coefficient), and molecular "fit" (stereochemistry) at the active site. These chemical characteristics ultimately affect absorption, distribution, excretion (in the case of plants, compartmentalization in a vacuole), bioactivation, and inactivation. Alterations in the basic structure of a drug or plant growth regulator can also affect the dose required to induce a particular biological response. Diphenhydramine, a highly flexible molecule, has both anticholinergic and antihistaminic action.[16] Introduction of a *t*-butyl group in the ortho position (2-position) results in a high anticholinergic and a low antihistaminic activity, while introduction of a methyl group in the para position (4-position) results in high antihistaminic and a low anticholinergic activity.[17,18] Studies with a series of substituted dinitroaniline herbicides on inhibition of tobacco callus tissue showed that substitution of an ethylpropyl group for an *N-sec*-butyl group on the amino nitrogen resulted in a 50-fold increase in inhibitory response.[19]

Binding characteristics of drugs to their complementary receptors can reveal important aspects of their behavior. Biologically active compounds react with some receptor molecule within the cell which then initiates a cascade of events leading to a response. Characteristics of biologically active molecules are a consequence of the chemical interactions with biochemical components of the organism (e.g., recognition of receptor sites). Among the drug and hormonal receptors that have been isolated and structurally identified in cellular membranes are cholinergic, nicotinic, muscarinic, α- and β-adrenergic subtypes, benzodiazepines, and the insulin family of receptors. Studies with plant systems and endogenous hormones have identified cytoplasmic/nuclear binding proteins which apparently stimulate the transcription of genes that are either directly or indirectly involved in the cell response to the plant hormones (auxins, cytokinins, etc.).[20] The data obtained for the cytoplasmic/nuclear auxin receptor agree with the model proposed for steroidal hormones.[20,21]

1.5 Direct-Acting Defense Chemicals — Mitotic Inhibitors and DNA Protectants

Since the discovery of vinca alkaloids in 1963, many of the major known antitubulin agents used in today's cancer chemotherapy arsenal are products of secondary metabolism. These "natural products" are probably defense chemicals that target and inhibit cell division in invading pathogens. Other phytochemicals such as resveratrol,[22] ellagic acid, beta-carotene, and vitamin E may possess antimutagenic and cancer-preventive activity.[23,24] Therefore, it is reasonable to hypothesize that plants produce chemicals that act in defense directly by inhibiting pathogen proliferation, or indirectly by disrupting chemical signal processes related to growth and development of pathogens or herbivores. Specific compounds or chemical families will be discussed in the following sections.

Colchicine is a poisonous tricyclic tropane alkaloid from the autumn crocus (*Colchicum autumnale*) and gloriosa lily (*Gloriosa superba*). This alkaloid is a potent spindle fiber poison, preventing tubulin polymerization.[25] Colchicine has been used as an effective anti-inflammatory drug in the treatment of gout and chronic myelocytic leukemia, but therapeutic effects are attainable at toxic or near toxic dosages. For this reason, colchicine and its analogs are primarily used as biochemical tools in the mechanistic study of new mitotic inhibitors.

Vinca alkaloids were discovered accidentally while evaluating the possible beneficial effects of periwinkle (*Catharanthus rosea*) in diabetes mellitus. Periwinkle produces about 30 chemical compounds in its alkaloid complex. The vinca alkaloids are cell-cycle-specific agents and, in common with other drugs, block cells in mitosis. The biological activities of these drugs can be explained by their ability to bind specifically to tubulin and block its polymerization into microtubules.[25] Through disruption of the microtubules of the mitotic apparatus, cell division is arrested at c-metaphase.[26] The inability to segregate chromosomes correctly during mitosis presumably leads to cell death.

Podophyllotoxin existence was recorded over 170 years ago in the U.S. Pharmacopeia in 1820. A resinous alcohol extract, obtained from the dried roots of the mandrake plant or Mayapple (*Podophyllum peltatum*) was used by native Americans and the colonists as a cathartic, an anthelmintic, and as a poison. Mandrake was identified as having a local antitumor effect as early as 1861. Two semisynthetic glycosides (etoposide and teniposide) of the active principle, podophyllotoxin, have been developed and show therapeutic activity in several human neoplasms, including pediatric leukemia, small-cell carcinomas of the lung, testicular tumor, Hodgkin's disease, and large-cell lymphomas.[27] Etoposide and teniposide are similar in their actions and in the spectrum of human tumors affected, but do not arrest cells in mitosis; rather, these compounds form a ternary complex with topoisomerase II and DNA. This complex results in double-stranded DNA breaks. Strand passage and resealing of the break that normally follow topoisomerase binding to DNA are inhibited by etoposide and teniposide. Topoisomerase remains bound to the free end of the broken DNA strand, leading to an accumulation of DNA breaks and cell death.[28]

Camptothecin (CPT) and its analogs are aromatic, planar alkaloids that are found in a very narrow segment of the plant kingdom. Potent antitumor activity of CPT was first discovered serendipitously in 1958 in fruit extracts from *Camptotheca acuminata*. The compound was isolated and the structure elucidated by Wall et al.[29] Camptothecin and its many analogs have a pentacyclic ring structure with only one asymmetric center in ring E, the pyridone ring D moiety, and the conjugated system linking rings A, B, C, and D.[30,31] Initial Phase I and II trials with CPT and topotecan have shown that responses have been obtained in the treatment of lung, colorectal, ovarian, and cervical cancers. CPT is a cytotoxic plant alkaloid that has a broad spectrum of antitumor activity. The drug is highly specific and kills cells selectively in the S phase. CPT inhibits both DNA and RNA synthesis; it produces a large number of single-stranded breaks in the presence of DNA topoisomerase I. CPT interferes with the breakage–reunion reaction of mammalian DNA topoisomerase I by trapping the key intermediate.[28] It appears that CPT causes arrest of the DNA replication fork that may be largely responsible for the termination of cellular processes. The presence of the α-hydroxy lactone moiety is one of several essential structural requirements for activity of CPT and its analogs.

Paclitaxel. The toxic properties of the yew have been known for at least 2000 years, but it was not until 1964 when Monroe Wall's group began working with bark extracts from the pacific yew (*Taxus brevifolia*) that its anticancer activity was demonstrated.[32] Paclitaxel (Taxol, now a patented trademark of Bristol-Myers Squibb) may be one of the most successful anticancer drugs of the decade. Taxol, more than most plant-derived medicines, exemplifies both the promise and the problems of natural product drug development (solubility and supply). Once scientists discovered the unique mechanism of action of Taxol and demonstrated its success in treating refractory ovarian cancer, Taxol became a focal point of conflict between human survival and natural resource exploitation. Paclitaxel is a diterpenoid compound that contains a complex taxane ring as its nucleus. Paclitaxel has undergone initial phases of testing in patients with metastatic ovarian and breast cancer; it has significant activity in both diseases. Early trials indicate significant response rates in lung, head and neck, esophageal, and bladder carcinomas. Paclitaxel binds specifically to the β-tubulin

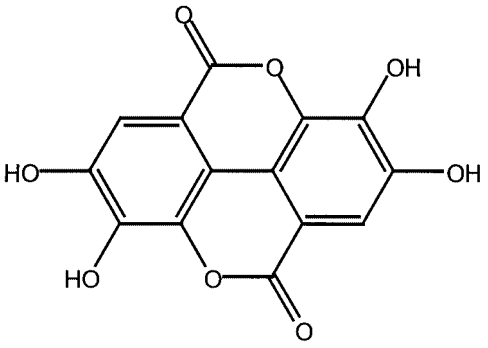


FIGURE 1.1
Ellagic acid is an astringent, hemostatic, antioxidant, antimutagenic, and possibly an antineoplastic agent from strawberries, raspberries, grapes, walnuts, and pecans. Its human dietary role in cancer prevention is uncertain and *in planta* function is unknown.

subunit of microtubules and appears to antagonize the disassembly of this key cytoskeletal protein, resulting in bundles of microtubules and aberrant structures and an arrest of mitosis.[33,34]

Ellagic Acid, a phenolic glucose derivative of castalagin, is the lactone form of a gallic acid dimer that occurs in plants, fruits, and nuts either in a free or conjugated form (Figure 1.1). Ellagic acid is present in high concentrations in walnuts and pecans and in fruits such as strawberries and raspberries.[35] Stoner[36] proposed that when fruits and nuts are consumed by humans, the glucose moieties of ellagitannins are probably removed by enzymatic activity in the digestive system, thus "freeing up" ellagic acid for absorption. Numerous derivatives of ellagic acid, formed through methylation, glycosylation, and methoxylation of its hydroxyl groups, exist in plants. These differ in solubility, mobility, and activity in plant as well as in animal systems.[37,38] The role of dietary ellagic acid in tumor suppression appears to be related to its antioxidant activities and activation of endogenous detoxification mechanisms. Both antioxidant and detoxification activities may be mediated by the quinone forms of ellagic acid. Previous interest in ellagic acid was largely due to its use in fruit juice processing and wine manufacturing. More recently, however, interest has focused on ellagic acid as a regulator of the plant hormone indole acetic acid, insect deterrent, blood-clotting agent, and anticarcinogen.[36,38,39]

In our continuing interests in natural product discovery,[40,41] ellagic acid and an extract of fruit from *Melia volkensii* (*MV*-extract) were screened for inhibition of *Agrobacterium tumefaciens*–induced tumors using the potato disk assay.[42] This bioassay is useful for the examination of plant extracts and purified compounds which inhibit crown gall tumors (a plant neoplastic disease) that may have potential human anticancer activity.[43,44]

Antitumor activity of ellagic acid and *MV*-extract were compared with that of CPT using the potato disk assay described by Galsky and Wilsey[45] and modified by Ferrigni et al.[46] Ellagic acid and *MV*-extract show dose-dependent activity against *A. tumefaciens*-induced tumors (Figure 1.2). Inhibition of tumor formation by CPT was similar for all doses tested and is consistent with its potent anticancer activity. Ellagic acid had greater antitumor activity at each concentration when compared with *MV*-extract, but had significantly less activity when compared with that of CPT. These data are consistent with the literature which states that ellagic acid may inhibit the initiation stage of carcinogensis that takes place in humans.[47]

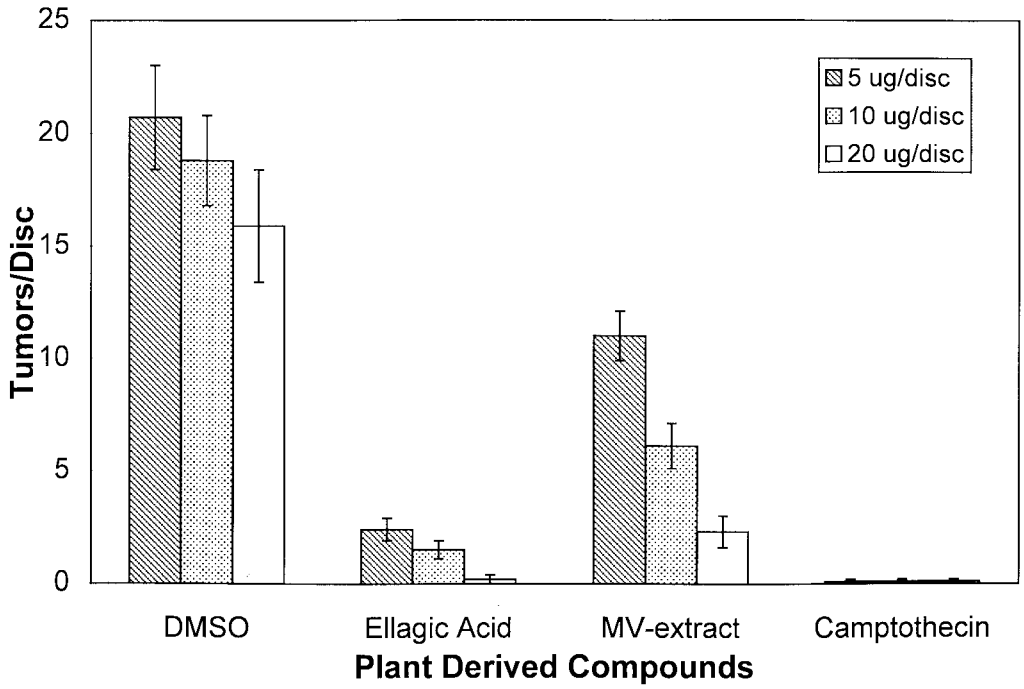


FIGURE 1.2
Tumor inhibition at three levels of Camptothecin (CPT), ellagic acid, and *MV*-extract tested in the *Agrobacterium tumefaciens*–induced tumor system. DMSO was used in the same concentrations as that used to test its respective dosage for each test compound. Error bars are indicative of ±1 standard error, $n = 15$.

1.6 Indirect-Acting Defense Chemicals — Fatty Acid Inhibitors and Signal Transduction

Plant resistance to pathogens is considered to be systemically induced by some endogenous signal molecule produced at the infection site that is then translocated to other parts of the plant.[48] Search and identification of the putative signal is of great interest to many plant scientists because such molecules have possible uses as "natural product" disease control agents. However, research indicates that there is not a single compound but a complex signal transduction pathway in plants which can be mediated by a number of compounds that appear to influence arachidonate metabolism. In response to wounding or pathogen attack, fatty acids of the jasmonate cascade are formed from membrane-bound α-linolenic acid by lipoxygenase-mediated peroxidation.[49] Analogous to the prostaglandin cascade in mammals, linolenic acid is thought to participate in a lipid-based signaling system where jasmonates induce the synthesis of a family of wound-inducible defensive proteinase inhibitor genes[50] and low- and high-molecular-weight phytoalexins such as flavonoids, alkaloids, terpenoids.[51,52]

Fatty acids are known to play an important role in signal transduction pathways via the inositol phosphate mechanism in both plants and animals. In animals, several polyunsaturated fatty acids like linolenic acid are precursors for hormones. Interruption of fatty acid

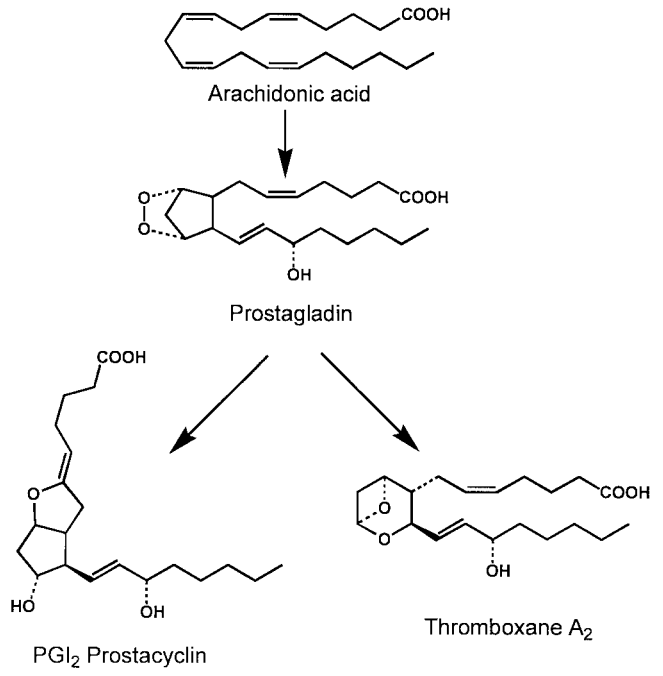


FIGURE 1.3
Arachidonic acid cascade.

metabolism produces complex cascade effects that are difficult to separate independently. In response to hormones, stress, infection, inflammation, and other stimuli, a specific phospholipase present in most mammalian cells attacks membrane phospholipids, releasing arachidonate. Arachidonic acid is parent to a family of very potent biological signaling molecules that act as short-range messengers, affecting tissues near the cells that produce them. The role of various phytochemicals and their ability to disrupt arachidonic acid metabolism in mammalian systems by inhibiting cyclooxygenase (COX-1 and COX-2) enzyme–mediated pathways is of major pharmacological importance.

Eicosanoids which include prostaglandins, prostacyclin, thromaboxane A2, and leukotrienes are a family of very potent autocoid signaling molecules that act as chemical messengers with a wide variety of biological activities in various tissues of vertebrate animals. It was not until the general structure of prostaglandins was determined, a 20-carbon unsaturated carboxylic acid with a cyclopentane ring, that the relationships with fatty acids was realized. Eicosanoids are formed via a cascade pathway in which the 20-carbon polyunsaturated fatty acid, arachidonic acid, is rapidly metabolized to oxygenated products by several enzyme systems including cyclooxygenases[53] or lipoxygenases,[54,55] or cytochrome P450s[56] (Figure 1.3). The eicosanoids maintain this 20-carbon scaffold often with cyclopentane ring (prostaglandins), double cyclopentane ring (prostacyclin), or oxane ring (thromboxanes) modifications. The first enzyme in the prostaglandin synthetic pathway is prostaglandin endoperoxide synthase, or fatty acid cyclooxygenase. This enzyme converts arachidonic acid to unstable prostaglandin intermediates. Aspirin, derived from salicylic acid in plants, irreversibly inactivates prostaglandin endoperoxide synthase by acetylating an essential serine residue on the enzyme, thus producing anti-inflammatory and anticlotting actions.[57]

Jasmonic acid is an 18-carbon pentacyclic polyunsaturated fatty acid derived from linolenic acid, plays a role in plants similar to arachidonic acid,[58] and has a structure similar to

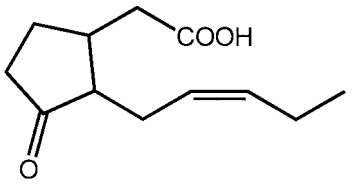


FIGURE 1.4
Jasmonic acid in plants plays a similar role to arachidonic acid in animals.

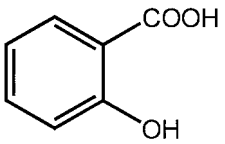


FIGURE 1.5
Salicylic acid is an important signal molecule inducing plant responses to pathogens.

the prostaglandins (Figure 1.4). It is synthesized in plants from linolenic acid by an oxidative pathway analogous to the eicosanoids in animals. In animals, eicosanoid synthesis is triggered by release of arachidonic acid from membrane lipids into the cytoplasm where it is converted into secondary messenger molecules. Conversion of linolenic acid through several steps to jasmonic acid is perhaps a mechanism analogous to arachidonate that allows the plant to respond to wounding or pathogen attack.[59] Linolenic acid is released from precursor lipids by action of lipase and subsequently undergoes oxidation to jasmonic acid. Apparently, jasmonic acid and its octadecanoid precursors in the jasmonate cascade are an integral part of a general signal transduction system that must be present between the elicitor–receptor complex and the gene-activation process responsible for induction of enzyme synthesis.[50,52,59] Closely related fatty acids that are not jasmonate precursors are ineffective in signal transduction of wound-induced proteinase inhibitor genes.[50] Arachidonic acid, eicosapentaenoic acid, and other unsaturated fatty acids (linoleic acid, linolenic acid, and oleic acid) are also known elicitors for sesquiterpenoid phytoalexins and induce systemic resistance against *Phytophthora infestans* in potato.[60]

Evidence is accumulating that salicylic acid plays an important role in pathogen response and plant resistance mechanisms (Figure 1.5). Jasmonic acid and salicylic acid appear to sensitize plant cells to fungal elicitors as they relay the signal in the induction of systemic acquired resistance. Acetylsalicylic acid (aspirin) inhibits the wound-induced increase in endogenous levels of jasmonic acid,[50] a response similar to the inhibition of prostaglandins. Both compounds induce resistance to plant pathogens and induce the synthesis of pathogenesis-related proteins.[61] Salicylic acid is an important endogenous messenger in thermogenesic plants.[62] Exogenous application of salicylic acid and aspirin to plants elicits a number of responses, one of which is blocking of the wound response.[61,63] It appears that polyunsaturated fatty acids derived from lipid breakdown (peroxidation), perhaps induced by wounding (injury) or in response to microbial invasion, may play important roles in signal transduction in many different organisms. This pathway may also prove to be a target site for control and protection not only of plants, but for new pharmaceuticals with quite specific activity. Toxicity of both synthetic and naturally occurring chemicals in biological systems frequently involves lipid peroxidation. Free radical production and subsequent actions are involved in mechanisms of herbicide action in plants as well as in other systems.

Increasing evidence suggests that plant cellular defenses may be analogous to "natural" immune response of vertebrates and insects. In addition to cell structural similarities, plant and mammalian defense responses share functional similarities. In mammals, natural immunity is characterized by the rapid induction of gene expression after microbial invasion. A characteristic feature of plant disease resistance is the rapid induction of a hypersensitive response in which a small area of cells containing the pathogen are killed. Other aspects of plant defense include an oxidative burst leading to the production of reactive oxygen intermediates (ROIs), expression of defense-related genes, alteration of membrane potentials, increase in lipoxygenase activity, cell wall modifications, and production of antimicrobial compounds such as phytoalexins.[64]

In mammalian immune response, ROIs induce acute-phase response genes by activating the transcription factors NF-κB and AP-1 genes,[65] and salicylic acid may play a role in the expression of NF-κB-mediated transcription.[66] In plants, ROIs and salicylic acid regulate pathogen resistance through transcription of resistance gene–mediated defenses. Functional and structural similarities among evolutionarily divergent organisms suggest that the mammalian immune response and the plant pathogen defense pathways may be built from a common template.[67] We believe that similar biosynthetic processes involved in signaling pathogen invasion and stress in plants and animals may account for the physiological cross activity of various fatty acid intermediates and other pharmacologically active phytochemicals.

1.7 New Chemistries and Modes of Action

Strobilurins, inspired by a group of natural products produced by edible forest mushrooms that grow on decaying wood, are being developed by Zeneca, Ag Products as azoxystrobin (Figure 1.6) and kresoxim-methyl (Figure 1.7) by BASF. Naturally occurring antifungal compounds, strobilurin A and oudemansin A, provide the wood-inhabiting mushroom fungi *Strobilurus tenacellus* and *Oudemansiella mucida* with a competitive advantage against other fungi.[68] Azoxystrobin (β-methoxyacrylate) was selected from 1400 compounds synthesized by Zeneca based on these naturally occurring antifungal products. Azoxystrobin had high levels of fungicidal activity, a broad-spectrum activity, low mammalian toxicity, and a benign environmental profile. *In vivo* greenhouse trials demonstrated LC_{95} values below 1 mg AI/l (active ingredient/liter) and broad-spectrum activity against important diseases caused by ascomycete, basidiomycete, deuteromycete, and oomycete plant pathogens. Strobilurins possess a novel mode of action by inhibiting mitochondrial respiration through prevention of electron transfer between cytochrome b and cytochrome c_1,[69] by

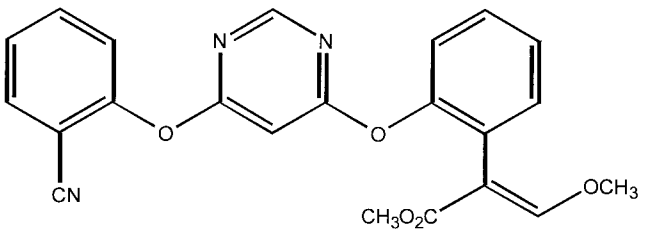


FIGURE 1.6
Azoxystrobin (β-methoxyacrylate) is one of 1400 synthesized from the lead compound, strobilurin A.

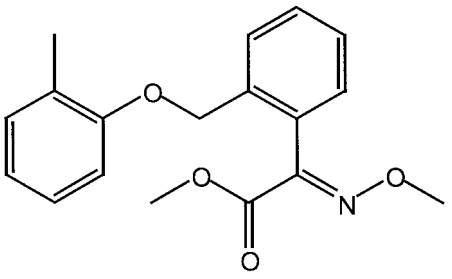


FIGURE 1.7
Kresoxim-methyl is based on the same strobilurin A lead compounds in which variations of the methoxyacrylate moiety produced the methoxyiminoacetate pharmacophore.

binding to the Q_o-site on cytochrome b.[70,71] Because of their novel mode of action, these compounds will offer control of pathogens resistant to other fungicides. Strobilurins have both disease preventative and curative properties and are active against spore germination, mycelial growth, and sporulation. More importantly, these compounds appear to be environmentally friendly. Azoxystrobin application rates as low as 200 g AI/ha have typically given control of potato late blight (*Phytophthora infestans*) and show low acute mammalian toxicity because fungal toxicity is not linked to mammalian toxicity. Knowledge of structural configuration and conformation and biological properties of strobilurin A has allowed the preparation of analogs in which both fungicidal activity and photostability have been improved. The importance and future of strobilurins as a new class of fungicides is seen by the fact that 21 companies have filed 255 patent applications primarily for use as fungicides.[69]

Spinosyns are a group of naturally occurring pesticidal compounds produced by the actinomycete *Saccharopolyspora spinosa* that were isolated from soil collected at a sugar mill rum still[72] (Figure 1.8). This group of macrolides, originally discovered by Eli Lilly scientists in the search for new pharmaceuticals,[11] led to the discovery of more than 20 spinosyns and development of a new chemical class, spinosyns.[73] Two spinosyns are being commercially developed by DowAgro under the label name of Conserve SC for insect control in turf and ornamentals. Conserve or spinosad (common name) is composed of the two most active macrocyclic lactones in a mixture of 85% spinosyn A and 15% spinosyn D.[74]

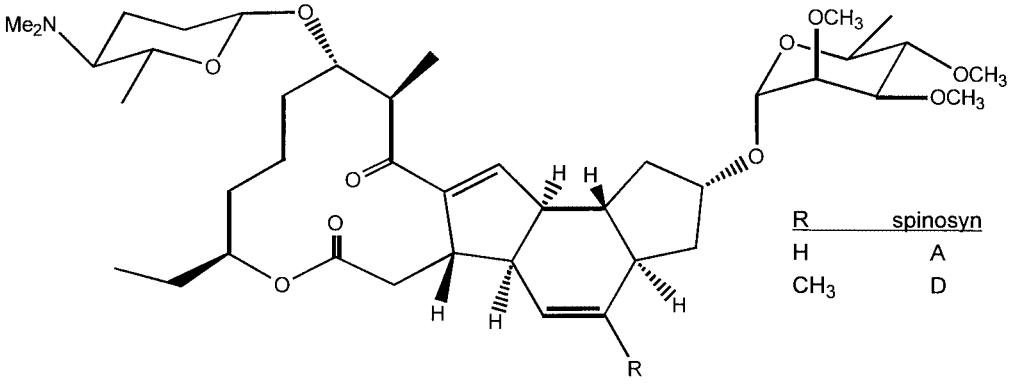


FIGURE 1.8
Spinosyn A and D are new natural product–based pesticidal macrolides originally discovered by Eli Lilly scientists in search for new pharmaceuticals.

Spinosyns act as both a contact and a stomach poison in insects, but are about five times more active orally in some species of insects such as the tobacco budworm (*Heliothis virescens*). Because spinosyns have a high efficacy and are especially active against a variety of lepidopterous pests,[75] Conserve is active at very low rates. Low rates of 0.08 lb/acre will control sod webworms (*Pediasia* sp.) and small armyworms (*Spodoptera mauritia*), a midrate of 0.27 lb/acre will control small cutworms (*Agrostis ipsilon*), and a high rate of 0.4 lb/acre will control large cutworms (*Agrostis ipsilon*) and armyworms (*Spodoptera mauritia*).[76] Spinosyns degrade very rapidly in the environment and have residual activities comparable to pyrethroids. Other attributes such as a unique mode of action, minimum impact on beneficial insects, low mammalian and nontarget toxicity, and rapid degradation by photolysis will make the spinosyn class of newly released natural products important pest controls for turf and ornamentals.

1.8 Conclusions

Plants and microorganisms are a proven source of numerous pharmaceutical and agrochemical agents, and it is reasonable to believe that there are additional agents in existence that remain undiscovered. These "natural products" are probably defense chemicals targeting and inhibiting the cell division processes of invading plant pathogens.[77,78] Inhibition of pathogen-induced DNA alteration and mutation may influence mechanisms common to the etiology of both animal and plant disease. Therefore, phytochemicals available from food components may affect tumorigenesis in humans by altering cellular responses to genetic damage or mitogenic stimulants. Ellagic acid is only one of many polyphenolic substances available from certain fruits, and human *in vivo* bioactivity of these phytochemicals is still speculative. However, ellagic acid available from a raspberry puree is now being evaluated for its ability to inhibit colon cancer in human clinical trial patients (Nixon, personal communication). Study of fresh fruits for use in dietary prevention, intervention, and recovery of cancer is ongoing at the Hollings Cancer Center at the Medical University of South Carolina. This research should provide data and help clarify cancer benefits attributed to some phytochemicals for human patients.[79]

Plant pathologists and breeders have realized for decades that phytochemical defense comes at an ecological cost; there are trade-offs between defense (resistance) and productivity.[80] Plant defense strategies were summarized into the optimal-defense theory by McKey[81] and elaborated by Rhoades,[82] but simply stated, you don't get something for nothing; there is a cost to everything. Information presented in this chapter supports reason to investigate phytochemicals further as sources for new chemistry. It also demonstrates further linkages between plant pathology and pharmacognosy in the study of phytochemistry and plant-related defense mechanisms.

Future development of value-added crops, nutraceuticals, phytopharmaceuticals, genetically enhanced fruits and vegetables, replacement crops for tobacco, and plant sources for the rapidly expanding herbal medicine industry will fuel the growth of alternative agricultural crops for nontraditional uses. The need to support research in alternative agriculture for the U.S. can be appreciated by the fact that the herbal/nutritional supplement market alone is valued at approximately 2 billion nationwide and 15 billion worldwide with an annual increase of 15%. Although the vast majority of the plant material is either collected from wild populations or grown outside the U.S., this situation provides U.S. growers with a major opportunity for expansion into alternative agricultural crops. Humanity's future

success in discovery and development of useful natural products will depend on knowledge and understanding of the diverse roles that phytochemicals play in the natural world and, of course, a healthy dose of serendipity.

References

1. Edmunds, M. *Defense in Animals*, Longman, Harlow, London, 357, 1974.
2. Blum, M.S. *Chemical Defenses of Arthropods,* Academic Press, New York, 562, 1981.
3. Harborne, J.B. *Introduction to Ecological Biochemistry,* 3rd ed., Academic Press, New York, 325, 1988.
4. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J.D. *Molecular Biology of the Cell*, 2nd ed., Garland, New York, 33, 1989.
5. Fritz, R.S. and Simms, E.L. *Plant Resistance to Herbivores and Pathogens,* The University of Chicago Press, Chicago, 1992.
6. Fenical, W. In *Alkaloids: Chemical and Biological Perspectives,* S.W. Pelletier, Ed., John Wiley & Sons, New York, 4, 1986, 275-330.
7. Rosenthal, J. and Janzen, D. *Herbivores: Their Interaction with Secondary Plant Metabolites,* Academic Press, New York, 718, 1979.
8. Duffey, J. *Annu. Rev. Entomol.,* 25, 447-477, 1980.
9. Svoboda, G.H. *Lloydia,* 24, 173-178, 1961.
10. Neuss, N. and Neuss, M.N. In *The Alkaloids,* Brossi, A. and Suffness, M., Eds., Academic Press, New York, 1990, Vol. 37, 229-239.
11. Kirst, H.A., Michel, K.H., Mynderase, J.S., Chio, E.H., Yao, R.C., Nakasukasa, W.M., Boeck, L.D., Occlowitz, J.L., Paschal, J.W., Deeter J.B., and Thompson, G.D. In *Synthesis and Chemistry of Agrochemicals III*, Baker, D.R., Fenyes, J.G., and Steffens, J.J., Eds., ACS Symposium Series No. 504, Amercian Chemical Society, Washington, D.C., 1992, 214-225.
12. Thinamm, K.V. *Hormone Action in the Whole Life of Plants,* University of Massachusetts Press, Amherst, 1977, 301.
13. Duke, S.O. *Rev. Weed Sci.,* 2, 16-44, 1986.
14. Sine, K.E. and Brown, T.M. *Principles of Toxicology,* CRC Press, Boca Raton, FL, 1996, 154.
15. Naylor, A.W. In *Herbicides: Physiology, Biochemistry, Ecology,* Audus, L.J., Ed., Academic Press, New York, 1976, chap. 1, 397-426.
16. Harms, A.F. and Natua, W.Th. *J. Med. Pharm. Chem.,* 2, 7, 1960.
17. Ariens, E.J. *Arzneim.-Forsch.*, 16, 1376, 1966.
18. Ariens, E.J. *Drug Design,* Academic Press, New York, 1971, 231.
19. Huffman, J.B. and Camper, N.D. *Weed Sci.,* 26, 527-530, 1978.
20. Libbenga, K.R. and Mennes A.M. In *Plant Hormones: Physiology, Biochemistry and Molecular Biology,* Davies, P.J., Ed., 2nd ed., Kluwer Academic Publishers, Boston, 1995, 272-297.
21. Trewavas, A. and Gilroy, S. *Trends Genet.,* 7, 356-361, 1991.
22. Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W.W., Fong, H.H.S., Farnsworth, N.R., Kinghorn A.D., Mehta, R.G., Moon, R.C., and Pezzuto, J.M. *Science*, 275, 218-220, 1997.
23. Balandrin, M.F., Kinghorn, A.D., and Rarnsworth, N.R. In *Human Medicinal Agents from Plants*, Kinghorn, A.D. and Balandrin, M.F., Eds., ACS Symposium Series No. 534, American Chemical Society, Washington, D.C., 1993, 2-12.
24. Pezzuto, J.M. In *Human Medicinal Agents from Plants*, Kinghorn, A.D. and Balandrin, M.F., Eds., ACS Symposium Series No. 534, American Chemical Society, Washington, D.C., 1993, 205-215.
25. Lu, M.C. In *Cancer Chemotherapeutic Agents*, Foye, W.O., Ed., American Chemical Society, Washington, D.C., 1995, 345-368.
26. Chabner, B.A., Allegra, C.J., Curt, G.A., and Calabresi, P. In *The Pharmacological Basis of Therapeutics,* Hardmand, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., and Gilman, A.G., Eds., McGraw-Hill, New York, 1996, 1233-1287.

27. Lee, K. In *Human Medicinal Agents from Plants*, Kinghorn, A.D. and Balandrin, M.F., Eds., ACS Symposium Series No. 534, American Chemical Society, Washington, D.C., 534, 1993, 170-190.
28. Sengupta, S.K. In *Cancer Chemotherapeutic Agents*, Foye, W.O., Ed., American Chemical Society, Washington, D.C., 1995, 205-239.
29. Wall, M.E., Wani, M.C., Cook, C.E., Palmer, K.H., McPhail, A.T., and Sim, G.A. *J. Am. Chem. Soc.*, 88, 3888-3890, 1966.
30. Wall, M.E. and Wani, M.C. In *Human Medicinal Agents from Plants*, Kinghorn, A.D. and Balandrin, M.F., Eds., ACS Symposium Series No. 534, American Chemical Society, Washington, D.C., 1993, 150-169.
31. Wall, M.E. and Wani, M.C. In *Cancer Chemotherapeutic Agents*, Foye, W.O., Ed., American Chemical Society, Washington, D.C., 1995, 293-310.
32. Kingston, D.G. In *Human Medicinal Agents from Plants*, Kinghorn, A.D. and Balandrin, M.F., Eds., ACS Symposium Series No. 534, American Chemical Society, Washington, D.C., 1993, 138-148.
33. Parness, J. and Horwitz, S.B. *J. Cell Biol.,* 91, 479-487, 1980.
34. Schiff, P.B., Fant, J., and Horwitz, S.B. *Nature,* 277, 665-667, 1979.
35. Rossi, M., Erlembacher, J., Zacharias, D.E., Carrell, H.L., and Iannucci, B. *Carcinogenesis,* 12, 1991, 2227-2232.
36. Stoner, G.D. *Proc. Annu. Mtg. Am. Strawberry Growers Assn.*, Grand Rapids, MI, 1989, 209-234.
37. Maas, J.L., Wang, S.Y., and Galletta, G.J. *HortScience,* 26, 66-68, 1991.
38. Maas, J.L., Galletta, G.J., and Stoner, J.D. *HortScience,* 26, 10-14, 1991.
39. Damas, J. and Remacle-Volon, G. *Tromb. Res.,* 45, 153-163, 1987.
40. Camper, N.D., Coker, P.S., Wedge, D.E., and Keese, R.J. *In Vitro Cell. Dev. Biol. Plant,* 33, 125-127, 1997.
41. Wedge, D.E., Tainter, F.H., and Camper, N.D. In *Phytochemicals and Health*, Gustine, D.L. and Flores, H.E., Eds., American Society of Plant Physiologists Series 15, American Soc. of Plant Physiologists, Rockville, MD, 1995, 324-325.
42. Mallico, E.J., Wedge, D.E., Fescemyer, H.W., and Camper, N.D. In *Proc. So. Nurserymen's Res. Conf.*, Atlanta, 42, 253-256, 1997.
43. McLaughlin, J.L. In *Methods in Plant Biochemistry.* Hostettmann, K., Ed., Academic Press, London, 6, 1991, 1-31.
44. McLaughlin, J.L., Chang, C., and Smith D.L. In *Human Medicinal Agents from Plants*, Kinghorn, A.D. and Balandrin, M.F., Eds., ACS Symposium Series No. 534, American Chemical Society, Washington D.C., 1993, 112-137.
45. Galsky, A.G. and Wilsey, J.P., *Plant Physiol.,* 65, 184-185, 1980.
46. Ferrigni, N.R., Putnam, J.E., Anderson, B., Jacobsen, L.B., Nichols, D.E., Moore, D.S., and McLaughlin, J.L., *J. Nat. Prod.,* 45, 679-685, 1982.
47. Pezzuto, J.M. In *Phytochemistry of Medicinal Plants*, Arnason, J.T., Mata, R., and Romeo, J.T., Eds., Plenum Press, New York, 1995, 29, 19-45.
48. Oku, H. *Plant Pathogenesis and Disease Control*, CRC Press, Boca Raton, FL, 1994, 193.
49. Vick, B.A. and Zimmerman, D.C. *Plant Physiol.,* 75, 458-461, 1984.
50. Farmer, E.E. and Ryan, C.A. *Plant Cell,* 4, 129-134, 1992.
51. Gundlach, H., Muller, M.J., Kutchan, T.M., and Zenk, M.H. *Proc. Natl. Acad. Sci. U.S.A.,* 89, 2389-2393, 1992.
52. Mueller, M.J., Brodschelm, W., Spannagl, E., and Zenk, M.H. *Proc. Natl. Acad. Sci. U.S.A.,* 90, 7490-7494, 1993.
53. Smith, W.L. *Am. J. Physiol.,* 268, F181-F191, 1992.
54. Samuelsson, B. *Science,* 20, 568-575, 1983.
55. Needleman, P., Turk, J., Jakschik, B.A., Morrison, A.R., and Lefkowith, J.B. *Annu. Rev. Biochem.,* 55, 69-102, 1986.
56. Fitzpatrick, F.A. and Murphy, R.C. *Pharmacol. Rev.,* 40, 229-241, 1989.
57. Insel, P.A. In *The Pharmacological Basis of Therapeutics,* Hardmand, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., and Gilman, A.G. Eds., McGraw-Hill, New York, 1996, 617-657.
58. Staswick, P.E. In *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, Davies, P.J., Ed., 2nd ed., Kluwer Academic Publishers, Boston, 1995, 179-187.

59. Farmer, E. and Ryan, C., *Proc. Natl. Acad. Sci. U.S.A.,* 87, 7713, 1990.
60. Cohen, Y., Gisi, U., and Mosinger, E. *Physiol. Mol. Plant Pathol.,* 38, 255-263, 1991.
61. Raskin, I. In *Plant Hormones: Physiology, Biochemistry and Molecular Biology,* Davies, P.J., Ed., Kluwer Academic Publishers, Boston, 1995, 188-205.
62. Raskin, I., Ehmann, A., Melander, W.R., and Meeuse, B.J.D. *Science,* 237, 1545-1556, 1987.
63. Doherty, H.M., Selvendran, R.R., and Bowles, D.J. *Physiol. Mol. Plant Pathol.,* 33, 377-384, 1988.
64. Dixon, R.A., Harrison, M.J., and Lamb, C.J. *Annu. Rev. Phytopathol.,* 32, 479-501, 1994.
65. Schreck, R. and Baeuerle, P.A. *Trends Cell Biol.,* 1, 39, 1991.
66. Kopp, E. and Ghosh, S. *Science,* 265, 956, 1994.
67. Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S.P. *Science,* 276, 726-733, 1997.
68. Clough, J.M. and Godfrey, C.R.A. *Chem. Brit.,* June, 466-469, 1995.
69. Godwin, V.M., Anthony, V.M., Clough, J.M., and Godrey, C.R.A. *Proc. Brighton Crop Prot. Conf. Pests and Diseases,* 1, 435-442, 1992.
70. Clough, J.M., Anthony, V.M., de Fraine, P.J., Fraser, T.E.M., Godfrey, C.R.A., Godwin, J.R., and Youle, D. In *Eighth International Congress of Pesticide Chemistry: Options 2000,* Ragsdal, N.N., Kearney, P.C., and Plimmer, J.R., Eds., ACS Conference Proceedings Series, Amercian Chemical Society, Washington, D.C., 1995, 59-73.
71. Gold, R.E., Ammermann, E., Kohle, H., Leinhos, G.M.E., Lorenz, G., Speakman, J.B., Stark-Urnau, M., and Sauter, H. In *Modern Fungicides and Antifungal Compounds,* Lyr, H., Russell, P.E., and Sisler, H.D., Eds., Intercept, Andover, MD, 79-92.
72. Mertz, F.P. and Yao, R.C. *Int. J. Sys. Bacteriol.,* 40, 34-39, 1990.
73. DeAmicis, C.V., Dripps, J.E., Hatton, C.J., and Karr, L.L. In *Phytochemicals for Pest Control*, Hedin, P.A., Hollingworth, R.M., Masler, E.P., Miyamoto, J., and Thompson, D.G., Eds., ACS Symposium Series 658, American Chemical Society, Washington, D.C., 1997, 144-154.
74. Sparks, T.C., Kirst, H.A., Mynderse, J.S., Thompson, G.D., Turner, J.R., Jants, O.K., Hertlein, M.B., Larson, L.L., Baker, P.J., Broughton, C.M., Busacca, J.D., Creemer, L.C., Huber, M.L., Martin, J.W., Nakatsukasa, W.M., Paschal, J.W., and Worden, T.W. In *Proceedings Beltwide Cotton Conferences*, Dugger, P. and Richer, D., Eds., National Cotton Council of America, Memphis, TN, 1996, 692-696.
75. Sparks, T.C., Thompson, G.D., Larson, L.L., Kirst, H.A., Jantz, O.K., Worden, T.W., Hertlein, M.B., and Busacca, J.D. In *Proceedings Beltwide Cotton Conferences,* Richter, D.A. and Armour, J., Eds., National Cotton Council of America, Memphis, TN, 1995, 903-907.
76. Conserve SC InfoSheet. Turf and ornamental insect pest control for lawn care and landscape professionals, DowAgro, 9330 Zionsville Road, Indianapolis, IN, 1997.
77. Feeny, P.P. Biochemical co-evolution between plants and their insect herbivores. In *Co-evolution of Animals and Plants*, Gilbert, L.E. and Raven, P.H., Eds., University of Texas, Austin, 1975, 246.
78. Feeny, P.P. *Recent Adv. Phytochem.,* 10, 1, 1976.
79. Nixon, D.W. *The Cancer Recovery Eating Plan.* Random House, New York, 1994, 452.
80. Zangerl, A.R. and Bazzaz, F.A. In *Plant Resistance to Herbivores and Pathogens,* Fritz, R.S. and Simms, E.L., Eds., The University of Chicago Press, Chicago, IL, 1992, 590.
81. McKey, D. *Am. Nat.,* 108, 305-320, 1974.
82. Rhoades, D.F. In *Herbivores: Their Interaction with Secondary Plant Metabolites*, Rosenthal, G.A. and Janzen, D.H., Eds., Academic Press, New York, 1979, 3-54.

2

Fractionation of Plants to Discover Substances to Combat Cancer

A. Douglas Kinghorn, Baoliang Cui, Aiko Ito, Ha Sook Chung, Eun-Kyoung Seo, Lina Long, and Leng Chee Chang

CONTENTS

2.1 Introduction
2.2 Potential Anticancer Agents
2.3 Potential Cancer Chemopreventive Agents
2.4 Conclusions
Acknowledgments
References

2.1 Introduction

In the U.S. for the year 1998, it is estimated that about 1,228,600 persons will be diagnosed with invasive cancer, and additionally about 1 million people will contract basal or squamous cancers of the skin. Furthermore, over 1500 persons per day (or over 560,000 Americans) will die in 1998 from cancer.[1] Plant natural products have had, and continue to have, an important role as medicinal and pharmaceutical agents, not only as purified isolates and extractives, but also as lead compounds for synthetic optimization.[2-6] For example, if cancer chemotherapeutic agents are considered, there are now four structural classes of plant-derived anticancer agents on the market in the U.S., represented by the *Catharanthus* (Vinca) alkaloids (vinblastine, vincristine, and vindesine), the epipodophyllotoxins (etoposide and teniposide), the taxanes (paclitaxel and docetaxel), and the camptothecin derivatives (camptotecin and irinotecan).[7-10] Plant secondary metabolites also show promise for cancer chemoprevention, which has been defined as "the use of non-cytotoxic nutrients or pharmacological agents to enhance intrinsic physiological mechanisms that protect the organism against mutant clones of malignant cells."[11] There has been considerable prior work on the cancer chemopreventive effects of constituents of certain culinary herbs, fruits, spices, teas, and vegetables, in which their ability to prevent the development of cancer in laboratory animals has been demonstrated.[12,13] Moreover, ellagic acid, isothiocyanates from *Brassica* species, and vanillin have been demonstrated mechanistically as carcinogenesis blocking (anti-initiating) agents, while curcumin, epigallocatechin gallate, limonene, and quercetin are effective carcinogenesis-suppressing (antipromotion/antiprogression) agents.[14] Clinical trials as cancer chemopreventive agents on plant products such as