AN ABSTRACT OF THE THESIS OF

Matthew W. Bernart for the degree of Doctor of Philosophy in Pharmacy presented on September 27, 1991.

Title: Natural Products from Tropical and Temperate Marine Algae

Abstract approved:___

William H. Gerwick

Following surveys of seaweeds in Oregon and the Caribbean, four algal species were selected for study on the basis of their crude extract biological activity or thin-layer chromatographic characteristics. Extracts were fractionated by normal phase chromatography, including high performance liquid chromatography (HPLC). Derivatives (methyl esters, acetates, and benzoates) of natural products were produced to facilitate isolation or structure elucidation efforts. Structure proofs utilized high-field nuclear magnetic resonance (NMR) spectroscopy, including hetero- and homo-nuclear two-dimensional experiments. Infrared and ultraviolet spectra, optical rotations, mass spectra, and circular dichroic spectroscopy were also employed.

The Puerto Rican red alga *Murrayella periclados* was found to contain (12*S*)-hydroxyeicosapentaenoic and (12*S*)-hydroxyeicosatetraenoic acids, two mammalian autacoids for which new biological activities are reported. Further eicosanoid metabolism was demonstrated by the isolation of (6*E*)-leukotriene B_4 and two diastereomers each of the insulin-release mediators hepoxilin B_3 and B_4.

The Oregon green alga *Acrosiphonia coalita* also was found to contain novel fatty acid-derived substances. Some of these contain an unprecedented structural feature, a conjugated trienal in which the aldehyde moiety branches off from the fatty acid chain. Exciton chirality studies of benzoate derivatives indicate that the alga introduces oxygenation in the 9*R*, 13*S*, and 16*S* positions on precursor polyunsaturated fatty acid chains.

The Oregon red alga *Laurencia spectabilis* was found to produce an equilibrium mixture of (±)-2-hydroxy-2-methyldihydrofuran-3-one and 5-hydroxy-2,3-pentanedione, which dimerizes on silica gel to produce spiro-bis-pinnaketal, a compound which was previously reported from *L. pinnatifida* and is probably an artifact of isolation.

The Oregon red alga *Prionitis lanceolata* displays high levels of apparent tryptophan metabolism. Never before isolated from a plant or marine source, 3-(hydroxyacetyl)indole, active at 10^{-10} M in the lettuce-seedling root elongation assay, was isolated in 0.07% yield along with indole-3-carboxaldehyde (0.5%) and indole-3-carboxylate (0.04%).

Natural Products from Tropical and Temperate Marine Algae

by

Matthew W. Bernart

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed September 27, 1991

Commencement June 1992

APPROVED:

Redacted for Privacy

Professor of Pharmacy in charge of major

Redacted for Privacy

Dean of College of Pharmacy

Redacted for Privacy

Dean of Graduate School

Date thesis is presented September 27, 1991

Typed by researcher for Matthew W. Bernart

ACKNOWLEDGEMENTS

I thank my advisor Dr. William H. Gerwick for the opportunity to work in his laboratory and for his moral and financial support. Not only did he teach me most of what I needed to know in order to perform this research; but he also helped me to develop an appreciation of marine natural products and scientific literature in general, as well as an awareness of what makes good science. Without him, it is unlikely that I would have ever found my calling and my life's work. I would also like to thank the members of my graduate committee for their valuable time and input. Dr. John H. Block co-ordinated biochemistry lectures with me and provided kind and meaningful feedback on content and style. In and away from his class, Dr. David J. Carlson stimulated my interests in marine ecological biochemistry. Dr. George H. Constantine offered encouragement and good cheer, especially during times when I was encountering misfortune in and away from the laboratory. Dr. Kevin P. Gable helped with NMR experiments and provided substantive editorial comments for improving this thesis. Through his course, Dr. Stephen J. Hawkes provided a theoretical and practical guide to the fascinating art and science of chromatography. Dr. Rosemary Wander showed genuine interest in these projects and volunteered her perspectives on the life of an academic researcher.

I would like to take this opportunity to thank other people who supported this effort, first of all to my past and present lab-mates. You know who you are and what you contributed. I thank the College of Pharmacy for and its staff for helping me through all these years. In Agricultural Chemistry, Brian Arbogast and Don Griffin provided us with mass-spectral services. I salute Rodger Kohnert of the Department of Chemistry for helping me to run many different NMR experiments. Dr. W. Curtis Johnson and Jeannine Lawrence in Biochemistry & Biophysics provided CD spectra. Don Unger at Kerr Library helped immensely with the literature searches for my thesis work. I appreciate the help and interest of phycologists Gayle Hansen, Eric Henry, and the late Harry K. Phinney. I thank Syntex Research Corporation and Dr. C.R. Pace-Asciak for testing some of our

compounds. Thanks also to Dr. Mats Hamberg for his collaborative efforts. George G. Whatley was responsible for much of the initial work on the *Acrosiphonia* project. Dr. Jon Clardy, E.E. Corcoran, and A.Y. Lee provided crystal structure data for the *Laurencia* work. Dr. John Cardellina and Michael Raub provided the lettuce-seedling bioassay for the *Prionitis* chapter. Dr. Terri Lomax is appreciated for her input on that project as well.

During my graduate program, I was supported by graduate research assistantships from Oregon Sea Grant. I also acknowledge support from N.L. Tartar Research Fellowships.

My brothers and sister, and especially my parents, Dr. William F. Bernart and Cynthia Wallace Bernart are to be thanked for their unflagging support. Though they were 3000 miles away, their love transcended the distance.

My cousin, Sam Davidson, is to be recognized as the only reason that I ever came to Corvallis in the first place. Here's to ya', Sammy.

Finally, I would like to thank my wife, Caryn, for helping me to get through my graduate program. Caryn encouraged me to continue on when I was on the verge of quitting. She also bore me two wonderful sons, Bryan Patrick Bernart and Logan Matthew Bernart. I thank them for many tender moments and giving me strength to do what had to be done.

TABLE OF CONTENTS

CHAPTER I: GENERAL INTRODUCTION AND LITERATURE REVIEW 1

CHAPTER II: EICOSANOID NATURAL PRODUCTS FROM THE TROPICAL RED ALGA *MURRAYELLA PERICLADOS* (C.Ag.) SCHMITZ 33

ABSTRACT 33

INTRODUCTION 34

RESULTS AND DISCUSSION 36

EXPERIMENTAL 52

CHAPTER III: UNPRECEDENTED OXYLIPINS FROM THE OREGON GREEN ALGA *ACROSIPHONIA COALITA* 71

ABSTRACT 71

INTRODUCTION 72

RESULTS AND DISCUSSION 74

EXPERIMENTAL 94

CHAPTER IV: LAURENCIONE, A HETEROCYCLE FROM THE OREGON RED ALGA *LAURENCIA SPECTABILIS* 119

ABSTRACT 119

INTRODUCTION 120

RESULTS AND DISCUSSION 121

EXPERIMENTAL 124

CHAPTER V: 3-(HYDROXYACETYL)INDOLE, A PLANT GROWTH REGULATOR FROM THE OREGON RED ALGA *PRIONITIS LANCEOLATA* 133

ABSTRACT 133

INTRODUCTION 134

RESULTS AND DISCUSSION 135

EXPERIMENTAL 137

BIBLIOGRAPHY 140

LIST OF FIGURES

Figure		Page
I.1	Chemical structures for Chapter I.	23
I.2	Mammalian pathways of eicosanoid metabolism.	30
I.3	Proposed mechanism of lipoxygenase reaction.	31
I.4	Allene oxide pathway to marine prostanoids.	32
II.1	Chemical structures for Chapter II.	59
II.2	12-Lipoxygenase metabolism in *Laurencia hybrida*, adapted in part from reference 198.	60
II.3	^{1}H NMR spectrum and proposed cyclized conformation of 12*S*-HETE (**8**).	61
II.4	^{1}H-^{1}H COSY of 12*S*-HEPE showing correlations between coupled protons.	62
II.5	^{1}H-^{1}H COSY of 6*E*-LTB$_4$ derivative (**9**) with labelled correlations between coupled protons.	63
II.6	^{1}H-^{1}H COSY of *threo* hepoxilin B$_4$ methyl ester (**13**) with labelled correlations between coupled protons.	64
II.7	Chemical ionization mass spectrum of trimethylsilyl ether derivative of hepoxilin B$_4$ methyl ester (**11** and **13**).	65
II.8	Newman projections and ^{1}H NMR spectra of two diastereomers of hepoxilin B$_4$ methyl ester.	66
II.9	Eicosanoid metabolism in *Murrayella periclados*.	67
III.1	Chemical structures for Chapter III.	103
III.2	Newman projection of derivative **2**.	104
III.3	^{1}H-^{13}C HETCOR of compound **3** showing expansion of the olefinic region.	105
III.4	^{1}H-^{1}H COSY of branched-chain trienal **4**.	106
III.5	^{1}H-^{13}C HETCOR of branched-chain trienal **4**.	107
III.6	Proposed biosynthesis of triene metabolites in *A. coalita*.	108
III.7	Newman projections of derivatives **10** & **11**.	110
III.8	Newman projection of derivative **13**.	111
III.9	^{1}H-^{1}H COSY of compound **14**.	112

III.10	Proposed formation of hydroxy-epoxy compounds in *A. coalita.*	113
III.11	Alternative formation of hydroxy-epoxy compounds in *A. coalita.*	114
IV.1	Chemical structures for Chapter IV.	128
IV.2	Acetylation of laurencione.	129
IV.3	ORTEP of Spiro-bis-pinnaketal (1).	130
V.1	Chemical structures for Chapter V.	139

LIST OF TABLES

Table		Page
II.1	NMR data for 12*S*-hydroxyeicosapentaenoic acid and two derivatives from *Murrayella periclados*.	68
II.2	Comparison of ^{1}H NMR spectrum of 12*S*-HEPE methyl ester with data for an unusual eicosanoid from *Laurencia hybrida*.	69
II.3	Algal eicosanoid enzyme inhibition assays (Syntex Research).	70
III.1	NMR data for two isomeric trienal alcohols from *Acrosiphonia*.	115
III.2	NMR data for three novel octadecanoid fatty acids isolated from *Acrosiphonia coalita* as methyl ester derivatives **4**, **5**, and **7**.	116
III.3	NMR data for two novel octadecanoid trienones isolated from *Acrosiphonia coalita* as methyl ester derivatives **6** and **8**.	117
III.4	NMR data for three hydroxy-epoxy octadecanoid fatty acids isolated from *Acrosiphonia coalita* as methyl ester derivatives **9**, **12**, and **14**.	118
IV.1	NMR data for laurencione ketal **5**, diketone **6**, and enone-diacetate **7**.	131
IV.2	NMR data for spirobispinnaketal **1** and its acetate **8**.	132

NATURAL PRODUCTS FROM TROPICAL AND TEMPERATE MARINE ALGAE

CHAPTER I. GENERAL INTRODUCTION AND LITERATURE REVIEW

This thesis is devoted to the investigation of previously undiscovered and biologically active chemicals found in tropical and temperate marine algae. The world's oceans are a vast resource of unique life forms, many of which are evolutionarily and biochemically distinct from their terrestrial counterparts. In recent years, investigators have found biologically active compounds in extracts of a variety of marine organisms. These substances, either as natural products or as chemical derivatives thereof, show potential as new drug molecules. New marine natural products, in addition to being a source of new antibiotics and anti-inflammatories, show utility for being pharmacological probes into the mechanisms of numerous human health and disease states.

The history of marine natural products goes back thousands of years. Tyrian purple, (1), a valuable indole-containing textile dye, was manufactured in the ancient Mediterranean city of Tyre from snail hypobranchial glands.[1,2] During the last three decades the field of marine natural products has blossomed. One reason for this has been a series of advances in the field of nuclear magnetic resonance (NMR) spectroscopy. Experiments utilizing this non-destructive technique are now routinely applied in ways that could only be dreamed of in years past. Moreover, progress in the fields of chromatography and mass spectrometry (MS) has made the tasks of purification and structure elucidation much more feasible. Additionally, following the Second World War, continued refinements by Cousteau and others in self-contained underwater breathing apparatus (SCUBA) technology have opened subtidal habitats to greater exploration.[3] Previously, specimens from these habitats were only accessible by dredge, and had escaped in-depth chemical study.

In the terrestrial environment, there exists an extensive history of phytochemistry, the study of plant chemistry. Many of today's drug molecules owe

all or part of their design to the biochemical processes of land plants. The terrestrial flora have been utilized for centuries as remedies for pain and disease as well as for a source of poisons. The science of pharmacognosy developed when chemists (as pharmacists were formerly known) found that active principles containing a desired pharmacological effect could be extracted from plants containing the sought-after biological activity. As just one example, the narcotic alkaloid morphine is still extracted from the opium poppy *Papaver somniferum*.

Obviously, all living organisms contain a variety of chemicals which may be extracted and purified. Those compounds which are structural units or are involved in the day-to-day vital functions of an organism, such as sugars, triglycerides, and sterols, are known as primary metabolites.[4] In micro-organisms, primary metabolites are usually produced during logarithmic growth, while secondary metabolites are generally produced later during the stationary phase. Although the boundary delineating primary and secondary is imprecise, secondary metabolites often give a competitive advantage to the producing organism, and include thousands of compounds which do not fit the definition for primary. Usually, primary metabolites are produced in much greater amounts than secondary metabolites by a particular organism. Because they are often toxic, antimicrobial, or display some other adaptive biological function, the secondary metabolites have received much attention from biosynthetic chemists and phytochemists in recent years.[5-7]

Secondary metabolism in the marine environment is quite different than on land, partly because of the ubiquitous supply of halogen ions in the ocean. Hydrocarbon, terpenoid, phenol and fatty acid metabolic pathways are found on both land and sea, but in the marine environment, the metabolites resulting from these pathways are more likely to be halogenated. Secondary metabolic pathways in the marine and terrestrial environments may have diverged since the time that land was first colonized by oceanic life. It would be premature to classify the marine algal biochemistry described in this thesis as the result of either primary or secondary metabolism, because in no instance has the function of these metabolites in the algae been discovered.

Chapters II and III respectively focus on modified polyunsaturated fatty acids (PUFAs) from a tropical red alga, *Murrayella periclados*, and a temperate-zone green alga, *Acrosiphonia coalita*. Chapter IV concerns a novel molecule named laurencione of unknown function, but unique chemical reactivity. Laurencione is produced in very large amounts (ca. 16% of the crude organic extract) by the Oregon red alga *Laurencia spectabilis*. Chapter V describes a plant growth regulatory compound and other putative tryptophan metabolites isolated from the Oregon red alga *Prionitis lanceolata*.

In this first chapter, after a brief introduction to the field of marine natural products and marine lipids, a review of PUFA metabolism will be presented. This review is necessary because Chapters II and III focus exclusively on known and novel fatty acid metabolites from the seaweeds. An overview of the relevance of fatty acid metabolism to human health will be presented, including a brief discourse on the enzymes involved in fatty acid oxygenation and modification in mammalian and human metabolic processes. PUFA chemical diversity in marine animals will be reviewed, followed by the marine algae, higher plants, and fungi. In order to review the literature pertinent to Chapter IV, which reports on novel chemistry from *Laurencia spectabilis*, an overview of the chemistry of the genus *Laurencia* will be presented. Finally, marine indole-containing metabolites will be reviewed in preparation for the final chapter, which reports probable tryptophan metabolites from *Prionitis lanceolata*.

Marine Natural Products and Marine Lipids.

An extensive body of literature is available which reviews the field of marine natural products. Scheuer has edited a series of reviews containing diverse chapters written by experts in the field.[8-10] Faulkner has comprehensively reviewed the marine natural products literature between 1977 and 1989 according to the taxonomical classification of the producing organisms, and has included structural and bio-activity data.[11-17]

Scheuer has observed that the current emphasis on searching the oceans for new anticancer drugs is unrelated to the marine environment, and is a result of today's research funding priorities.[18] That situation is slowly evolving into broader-spectrum investigations worldwide for different types of biological activities. Cardellina, now at the National Cancer Institute (NCI), has evaluated various attempts to discover and develop marine natural products as new agrochemical and pharmaceutical agents.[19] Rinehart reviewed his and others' work on a variety of different screening techniques applicable to marine organisms, including antimicrobial, antiviral, antitumor, immunoregulatory, cardioregulatory, neurotoxicity, and anti-inflammatory methods.[20] Currently, our laboratory is part of a drug-discovery group which includes researchers at the Universities of Oklahoma and California (Santa Cruz). In this project, screening at Syntex Research Corporation and at NCI is mostly devoted to anticancer assays, although some anti-inflammatory assays have been performed. The current state of the field was evaluated by marine pharmacologist Jacobs of the University of California at Santa Barbara, who remarked that "the sea holds so many medically important molecules that if we all worked 'til the end of our lives we would barely scratch the surface."[21]

One important area of the marine natural products discipline is the field of marine lipids. The lipids are a chemically diverse group of substances which are insoluble in water, but soluble in organic solvents.[22] In nutrition, lipids are generally thought of as triglycerides, fatty acids, phospholipids, and sterols, especially cholesterol. The metabolism of these lipids in humans, animals, plants, and micro-organisms has been studied extensively.[22-27] An encompassing handbook[28] of lipids features a dictionary section containing data and references for hundreds of lipid molecules, including many marine natural products. Considerable interest has been generated in the study of marine fatty acids and oils, their metabolism, and their effects on human health.[29,30] Reports in the literature have demonstrated that increased fish oil consumption improves inflammatory conditions such as psoriasis,[31] rheumatoid arthritis,[32] and cardiovascular disease[33] by decreasing the production of proinflammatory

metabolites in the body.[30] Unsubstantiated claims for fish oil-type fatty acid supplements include the relief of schizophrenic and manic-depressive syndromes along with the production of greater calm and emotional strength.[34] Clearly, a more comprehensive understanding of the marine lipids and their effects on human health would be appreciated by the scientific community and beneficial to the general public.

The major difference between the marine and terrestrial fatty acids is that the marine fatty acids are more unsaturated. Algae, the ultimate source of fish fatty acids, are rich in polyunsaturated fatty acids (PUFAs) such as arachidonate, (AA, **2**), an ω-6 PUFA, and eicosapentaenoate (EPA, **3**), an ω-3 PUFA.[22,35] (Omega nomenclature positions are determined by counting from the terminal methyl group (ω-1) of the fatty acyl chain until the first olefinic carbon is reached.) In the cultured red alga *Porphridium cruentum*, EPA is the predominant fatty acid under optimal growing conditions. However, when the growth rate of the alga was reduced by decreased light intensity or other adverse conditions, EPA was replaced by AA as the major PUFA.[36] Many other PUFAs are found in the algae, including 18-carbon chain length fatty acids of both the ω-3 and ω-6 series.[22] These algal PUFAs are incorporated into the lipids of herbivorous fish, and from there into carnivorous fish, such as salmon, which is renowned for its high ω-3 content. Although oceanic and freshwater fish are a rich source of PUFAs in the human diet, terrestrial plants can also supply these fatty acids or their precursors. A certain amount of PUFA in the diet is essential to humans and other animals. The essential fatty acids may include those which are unsaturated at the ω-3 as well as at the ω-6 position. These unsaturations cannot be accomplished in the animal cell, as animals lack the enzymes necessary to desaturate fatty acids beyond the Δ-9 position. Therefore, ω-3 and ω-6 fatty acids must ultimately come from plant fatty acid biosynthetic pathways. One reason that these fatty acids are essential to the health and well-being of the individual is that they are are transformed into a variety of important hormonal substances, some of which are discussed in the following section.

Enzymatic Transformations of Polyunsaturated Fatty Acids.

Although PUFAs are important structural lipids and energy sources, this review will focus on oxidative enzymatic transformations of PUFAs into biologically active metabolites. The nomenclature of these metabolites has been the subject of much interest and concern.[37-39] The term "eicosanoid", from the Greek root meaning twenty, commonly denotes the oxygenated metabolites of 20-carbon fatty acids. However, the some of the enzymes which enact the transformations of the 20-carbon fatty acids also accept different chain lengths as substrates. The term "oxylipin" has been proposed as an encompassing term for PUFA metabolites formed by reaction(s) involving one or more steps of mono- or di-oxygenase catalyzed oxygenation, thus including the eicosanoids as well as metabolites of different chain length.[40] The chemistry, biosynthesis, and pharmacology of this structure class has generated ever-increasing interest, especially in the last two decades. Thousands of articles are published in this field each year.[41]

In humans, there are three major enzyme cascades involved in PUFA oxidation: the cytochrome P_{450}, cyclooxygenase, and lipoxygenase pathways. Figure I.2 summarizes these three pathways, depicting AA as the fatty acid substrate, although other PUFAs may also enter the cascades. One or more of these pathways is found in almost every type of mammalian tissue studied. The metabolites of these pathways exert profound biological effects at very low concentrations in biological fluids, even down into the nanomolar range. The cytochrome P_{450} enzymes, a family of mono-oxygenases which contain a heme iron prosthetic group, introduce an oxygen atom at double bonds in PUFA substrates, usually yielding epoxy or hydroxy metabolites.[42] An example of P_{450} metabolism of arachidonate is (12*R*)-hydroxyeicosatetraenoate (12*R*-HETE, **4**). When arachidonate, (**2**), was incubated with kidney-derived cytochrome P_{450}, the 12*R*-HETE which resulted was found to inhibit Na^+/K^+ ATPase in the heart.[43] This type of ATPase enzyme is an ion pump which regulates osmotic balance and maintains ionic gradients in a multitude of animal and plant tissues. Later, 12*R*-

HETE was found in the skin lesions of psoriasis patients, and was found to be a chemoattractant for polymorphonuclear leukocytes, a type of white blood cell, in vitro.[44]

The cyclooxygenase pathway, which leads to the production of the prostaglandins, thromboxanes, and prostacyclin, was the first of the eicosanoid pathways to be studied. The enzyme which initiates this cascade is actually known as prostaglandin H (PGH) synthase, an enzyme which contains both the cyclooxygenase and peroxidase activities.[45] PGH is a reactive endoperoxide intermediate which is rapidly converted to the prostacyclin, thromboxane, and prostaglandin series of compounds. In mammals, these metabolites modulate many vital functions such as smooth muscle constriction, reproduction, sodium ion and urine excretion, broncho- and vaso-constriction, atherosclerosis, and thrombosis.[46,47] Recent discoveries have shown that the biological effects of many cyclooxygenase metabolites are receptor mediated.[48] Although prostaglandins have been isolated from marine organisms, it is generally accepted that the PGH synthase pathway is not operable in marine invertebrates.[49-51] In fact, most marine prostaglandins are formed by a distinctly different pathway, to be discussed later in this chapter, involving an allene oxide intermediate.

The route of oxylipin metabolism most apparent and detectable in the marine algae is the lipoxygenase pathway.[40,52,53] Lipoxygenase is a general term for a class of dioxygenases, all containing a non-heme iron prosthetic group, which enact PUFA transformations in both plants and animals. In 1947, a pure, crystalline lipoxygenase enzyme was isolated from soybeans.[54] Almost three decades later, Samuelsson, Hamberg and others discovered that lipoxygenase activity in human blood platelets gives rise to a family of biologically active metabolites.[55,56] Because lipoxygenases require a *cis, cis*-1,4-pentadienyl moiety, PUFAs are their preferred substrates. Corey and Nagata have presented experimental evidence that the soybean lipoxygenase reaction is an organoiron-mediated, concerted process.[57] Proton abstraction and electrophilic addition of Fe(III) to carbon occur as shown in Figure I.3. The organoiron intermediate, which is co-ordinated to the enzyme, allows the hydroperoxide product to be

formed via σ bond insertion of dioxygen. Prior to Corey's work, a radical mechanism had been proposed.[58] In either case, molecular oxygen is inserted antarafacially two carbons distant from the site of proton abstraction, resulting in a *cis, trans* conjugated diene system vicinal to a hydroperoxide moiety. This reactive allylic hydroperoxide may then be transformed into a variety of biologically important substances.

Lipoxygenases are typically classified by the number of the carbon atom, counting from the carboxyl terminus, at which the oxygen is introduced onto the fatty acid chain. The best-studied human lipoxygenases are the 5-, 12-, and 15-lipoxygenases, all of which typically utilize arachidonate and EPA as substrates. Among the products of these enzymes are the monohydroxy fatty acids, leukotrienes (LTs), and lipoxins. These metabolites play crucial roles in the mediation of inflammatory processes including asthma and psoriasis,[59-61] and may be involved in the modulation of neurotransmitter release.[62] Citing LTB_4 (**5**) as an example, evidence is mounting that these lipoxygenase metabolites act at receptors on effector cell membranes.[63]

Oxylipin Metabolism in Marine Invertebrates.

The natural products literature lacks any chemically oriented review of PUFA metabolism in marine invertebrates. The purpose of this section is to give a non-comprehensive, taxonomically oriented summary of some of the metabolites of these simple invertebrates, especially those exemplifying unusual chemical or biological phenomena. The general trend here will be to cover the evolutionarily simple animals first, and then considering the more advanced. Stanley-Samuelson has reviewed the physiological roles of PGs and other eicosanoids in marine and terrestrial invertebrates, including regulation of temperature, ion flux, reproduction, and cell aggregation.[64]

Given the physiological and biosynthetic interest in the eicosanoids, rarely has a single report garnered so much attention as the 1969 discovery by Weinheimer and Spraggins of 15*R*-PGA_2 (**6**) and its diester (**7**) in the gorgonian

coral *Plexaura homomalla* (Coelenterata).[65] Soon afterward, this coral was discovered to be a rich source of various other prostaglandins (up to 3% on a dry weight basis), including both C-15 isomers of PGA_2 and PGE_2 (**8**).[66,67] The 15*S*-isomer of PGA_2 lowers mammalian blood pressure, while the 15*R*-isomer lacks this activity. Mammalian PGE_2 is also of the 15*S* configuration, and it displays a host of biological activities at minute concentrations, including vasodilation, increased cardiac output, smooth muscle relaxation, inhibited gastric acid secretion, and the modulation of pain.[68] The ecological significance of prostaglandins in *Plexaura* was later explored by Gerhart, who found that either C-15 isomer of PGA_2 was an effective feeding deterrent against predatory reef fish.[69] In a survey of prostaglandin synthesis activity in various marine invertebrates, Morse and co-workers employed an arachidonate-dependent epinephrine oxidation assay.[70] By this method, many tropical coelenterates were identified as possible sources of prostaglandins. However, a later study found this method to be very unreliable and of doubtful validity.[71]

Based on an elegant series of biosynthetic experiments, prostaglandin-type cyclopentenone ring formation in *Plexaura*, as well as in other species of corals, is thought to proceed via the allene oxide pathway shown in Figure I.4.[50,51,72-74] Lipoxygenation of arachidonate at C-8 gives the 8*R*-hydroperoxy intermediate. Proton abstraction at C-9 allows rearrangement into a transient allene oxide. This reactive species may form an oxidopentadienyl cation which subsequently cyclizes to give the cyclopentenone moiety found in many marine prostaglandins.

Eicosanoid production is not restricted to the gorgonian corals. The Okinawan soft octocoral *Clavularia viridis*, a member of the order Stolonifera, produces a series of novel eicosanoids called the clavulones. These were originally isolated from the methanolic extract of the coral bodies, and were reported to exhibit anti-inflammatory activity.[75] The absolute stereochemistry of the clavulones, as represented by clavulone I (**9**), was determined by circular dichroic (CD) analysis of *para*-bromobenzoate derivatives.[76] Soon after the original clavulone paper appeared, another group reported the isolation of a series of "claviridenones" from the same organism which turned out to be identical

to the clavulones.[77] In an unfortunate duplication of efforts, this second group also deduced the absolute stereochemistry of the "claviridenones" by a similar strategy.[78] It has been recommended that the name "claviridenone" be retired for the b, c, and d members of that series, to be replaced by clavulones III, II, and I, respectively.[79]

Clavularia produces many structural variations on the clavulone theme, including cytotoxic clavulones containing a halogen at C-10 which inhibit the growth of a human promyelocytic leukemia (HL-60) cell line.[80,81] A structure-activity investigation of the clavulone series[82] allowed the following trends to be discerned: 1) a double bond or epoxide at C10-11 is required for maximal antiproliferative and cytotoxic effects, 2) a hydroxyl group of either stereochemical configuration at C-12 enhanced the antiproliferative and cytotoxic activity, 3) a halogen at C-10 potentiated both these activities (Cl = F > Br = I > H). Cytotoxic concentrations for the clavulone series are mostly within an order of magnitude of the IC_{50} (inhibitory concentration to 50% of the cell population) to the cancer cell line. Possibly, the clavulones could be considered as templates for the design of new anticancer drugs. In biosynthetic experiments using a cell-free *Clavularia* homogenate, Corey's group has found evidence that the clavulones also are formed from arachidonate via an allene oxide pathway.[83,84]

Hawaiian collections of another octocoral, *Telesto riisei*, by Scheuer's group resulted in the discovery of the punaglandins, one of which, punaglandin 3, was fifteen times more inhibitory towards L 1201 leukemia cell proliferation (IC_{50} = 0.02 μg/ml) than the corresponding clavulone.[85] Following chemical synthesis, the stereochemistry of punaglandins 3 (**10**) and 4 (**11**) has been revised from 12*S* to 12*R*.[86] It seems likely that the punaglandins 3 and 4 derive from eicosapentaenoate and arachidonate, respectively.

Because of difficulties in sponge taxonomy, and due to the diversity of symbiotic unicellular algae and other organisms which live within sponges, the origin of oxylipins from sponges is intriguing but unclear. Wells, while at the now defunct Roche Research Institute of Marine Pharmacology in Dee Why, Australia, isolated a unique peroxyketal called chondrillin (**12**) from a Great Barrier Reef

sponge of the genus *Chondrilla*.[87] A decade later, Crews' group re-isolated chondrillin, along with novel xestin A (13) and its C-6 diastereomer xestin B from the dichloromethane extract of Fijian *Xestospongia* sp.[88] Xestin A was ten times more potent in vitro against P388 murine leukemia cells (IC_{50} = 0.3 μg/ml). Xestin A was somewhat less active against lung, colon, and mammary tumor cells, while xestin B was inactive. These findings serve to illustrate that the stereochemistry of oxylipins may affect their biological activities. Evidently, these sponges have long-chain (22 and 24 carbon) fatty acids available for oxylipin metabolism. More recently, cytotoxic 18-carbon homologs of the xestins, as well as chondrillin, were isolated from the unrelated Okinawan sponge *Plakortis lita*.[89]

Molinski and Ireland reported a new cytotoxic and antimicrobial azacyclopropene, dysidazirine (14), from the Hawaiian sponge *Dysidea fragilis*.[90] Dysidazirine, the first example of a marine azacyclopropene, may be derived from sphingosine or possibly an 18-carbon fatty acid. Dysidazirine was toxic to L 1210 cancer cells and strongly inhibited the growth of the gram-negative pathogen *Pseudomonas aeruginosa* and the yeasts *Candida albicans* and *Saccharomyces cerevisiae* (4 μg/disk in the sensitivity disk bioassay).

Commonly encountered in Oregon and elsewhere, the sponge *Halichondria* is the last sponge to be considered in this discussion. A methanolic extract of Japanese *Halichondria okadai* was found to contain the novel eicosanoids halicholactone and neohalicholactone. Halicholactone was reported to be a weak inhibitor of guinea pig polymorphonuclear leukocyte 5-lipoxygenase.[91] An X-ray crystallographic study[92] later solved the relative stereochemistry of neohalicholactone (15), the ω-3 metabolite which ostensibly derives from EPA. Presumably, halicholactone incorporates the same stereochemical features as neohalicholactone, only in an arachidonate-derived carbon skeleton.

It is a giant step up the evolutionary ladder from corals and sponges to the gastropod opistobranch molluscs, yet the oxylipin metabolism seen in primitive creatures is apparently conserved in the more advanced organisms. The marine molluscs are renowned for their large neurons, rendering them desirable for dissections and neuroanatomical studies. In the late 1980's, Piomelli's group

uncovered evidence for the synthesis of second messengers via 12-lipoxygenase metabolism in the sensory neurons of the sea hare *Aplysia californica*.[93,94] Furthermore, 12-hydroperoxyeicosatetraenoate (12-HPETE, **16**) and its metabolite 12-ketoeicosatetraenoate (12-KETE, **17**) were shown to participate in the transduction of histamine responses in the *Aplysia* neuron.[95] Hepoxilin A_3 (**18**), a 12*S*-lipoxygenase derived mammalian insulin release mediator[96] and modulator of calcium transport,[97] produces slow hyperpolarization with increased membrane conductance in certain *Aplysia* neurons.[98] More advanced *Aplysia* eicosanoid metabolism was discovered in Japanese *A. kurodai*, which is reported to produce aplydilactone (**19**).[99] Aplydilactone was reportedly a weak inhibitor of phospholipase A_2 (PLA_2), an enzyme which releases arachidonate from membrane phospholipids.

The nudibranch mollusc *Tethys fimbria*, collected in the Bay of Naples, sequesters PGE_2 1,15-lactones (**20**) and PGE_3 1,15-lactone (**21**) in its mantle tissue.[100] It is postulated that these lactones are cyclooxygenase metabolites.[101] These PGE 1,15-lactones are biosynthesized from free PG in the mantle and are converted back into free PG's during autotomy,[102] a defensive tactic in which bodily appendages are shed when the organism is disturbed. Upon the discovery that PGF 1,15-lactones are also found in the same mollusc, as well as its egg masses, the Italian researchers have hypothesized that while the PGF-series lactones may serve a reproductive function. The PGE series may play a dual role, the lactones being agents of chemical defense while the free acids modulate muscle contractions.[103]

The phylum Echinoderma includes the starfish and sea urchins. Brash and co-workers discovered that various species of starfish synthesize 8*R*-HETE (**22**) from exogenous arachidonate.[104] Of the many lipoxygenase metabolites tested for bioactivity, including 8*S*-HETE, only 8*R*-HETE induced starfish oocyte maturation. Evidence showed that this was caused by a decrease in cyclic adenosine monophosphate levels and an increase in protein phosphorylation. Methyl esterified 8*R*-HETE was ten times less active than the free acid. The subsequent isolation of 8*R*-HETE and (8*R*)-hydroxyeicosapentaenoate (8-HEPE,

23) from the starfish *Patiria miniata* proved that these metabolites are formed from endogenous lipids in the starfish.[105] The sea urchin *Strongylocentrotus purpuratus* exhibits calcium-dependent 11*R*- and 12*R*-lipoxygenase activity which utilizes endogenous lipids as substrates.[106]

Arthropods are a diverse group of invertebrates with representatives on land and sea. Within this group, the ubiquitous marine barnacle causes millions of dollars worth of lost time and damage as it grows on ship bottoms and docks. As part of its reproductive cycle, the barnacle *Balanus balanoides* produces 10,11,12-triHETE (**24**) of undefined stereochemistry as its larval hatching hormone.[107] This metabolite might be formed by the opening of the epoxide of the 12-lipoxygenase metabolite hepoxilin B_4 (**25**)[108] with water. Recently, a series of similar trihydroxy PUFAs and monochlorodihydroxy metabolites were reported from the same species.[109] However, it was mentioned in this report that the chlorinated metabolites may actually be artifacts formed in the presence of seawater or chloroform during the extraction process.

Oxylipin Metabolism in Marine Algae.

In this section, a phylogenetic treatment of published research in the algal oxylipin field will be presented. Eicosanoids from the Rhodophyta (red algae) have been reviewed.[52] A more comprehensive compilation of oxylipin chemistry in the Rhodophyta, Phaeophyta (browns), Cyanophyta (blue-greens), and Chlorophyta (greens) is in press.[53]

The first report of prostaglandins in a plant was the discovery of PGs in aqueous extracts of the red alga *Gracilaria lichenoides*.[110] Since the original report, the taxonomy of this alga has been revised to *G. edulis (lichenoides)*.[111] PGE_2 was isolated with the aid of a biological assay which monitored its antihypertensive activity. $PGF_{2\alpha}$ (**26**), a major uterine PG, was also recovered from the algal extract. More recently, it was demonstrated that PGE_2 from Japanese *G. verrucosa* was responsible for "Ogonori" poisoning, a severe form of diarrhea occasionally affecting those who eat the alga.[112] In our laboratory, the

chloroform-methanol extract of *Gracilariopsis lemaneiformis* was found to contain a novel monogalactosyldiacylglyceride (MGDG, **27**) along with related new digalactosyldiglycerides (DGDGs).[113] The MGDGs and DGDGs are typically associated with chloroplast membranes. *G. lemaneiformis* also produces a series of hydroxylated PUFAs and related compounds.[114] Acetone powder enzyme preparations of the seaweed, suspended in phosphate buffer and incubated with arachidonate, produce 12*S*-HETE.[115] This unstable eicosanoid is best known for its inhibition of platelet aggregation[116] and for its stimulation of chemotactic activity in neutrophils,[117] a type of white blood cell. Thus far, the most ubiquitous eicosanoid, at least among the red algae, seems to be 12*S*-HETE.

Some of the first seaweeds examined for eicosanoid production were temperate and tropical specimens from the order Ceramiales. The Oregon red alga *Ptilota filicina* produces ptilodene (**28**), a novel inhibitor of 5-lipoxygenase and canine renal Na^+/K^+ ATPase.[118] A more detailed discussion of oxylipin chemistry in the Ceramiales is presented in Chapter III of this thesis. Algae in the family Delesseriaceae produce eicosanoids which are identical to known mammalian immunomodulators. The discovery of 12*S*-HETE in a Puerto Rican collection of *Platysiphonia miniata* constituted the first report of this compound from a plant source.[119] This alga, along with *Cottoniella filamentosa*, also from Puerto Rico, yielded an undetermined diastereomer of hepoxilin B_3 (**29**).[120] Like hepoxilin A_3, hepoxilin B_3 stimulates insulin release from pancreatic islet cells.[96] In contrast to some of the invertebrate oxylipins for which functions have been assigned, no physiological purpose has been conclusively proven for any of the algal metabolites of this structure class.

The intertidal red alga *Farlowia mollis* was the first of Oregon's Cryptonemiales to be examined in our laboratory for oxylipin content. Three farlowdiols (**30-32**), structurally related vicinal-diol PUFA metabolites, were isolated from the lipid extract.[121] A 2:3 mixture of farlowdiols **30** and **31** inhibited the 5-lipoxygenase and dog kidney Na^+/K^+ ATPase. Further, they stimulated superoxide anion production in isolated human neutrophils, a signal of the oxidative burst phase of the immune response. The stereochemistry shown for

farlowdiol **30** is that which Lumin and Falck recently determined by total synthesis.[122] Evidently, the farlowdiol structural unit (**30**) is incorporated as the acyl chains of *G. lemaneiformis* MGDG **27**. The structure of the 18-carbon farlowdiol (**32**) gives an indication that the putative algal lipoxygenase recognizes the methyl terminus of the PUFA substrate in order to determine the position of oxidation on the fatty acid chain.

From the family Weeksiaceae, *Constantinea simplex* produces a lactonized cyclopropyl eicosanoid, constanolactone (**33**),[123] of which an ω-3 homolog is likely to be the monomeric unit in the dimerized *Aplysia* eicosanoid, aplydilactone (**19**).[99] Because *Aplysia* eat mostly algae, it would be interesting if a functionalized eicosanoid precursor of dietary origin could be identified in this instance. A mixture containing lactonized and opened, methylated forms of constanolactone was weakly inhibitory toward PLA_2, as was aplydilactone. The co-occurrence in the alga of 12*S*-HETE and -HEPE makes 12-lipoxygenase biogenesis of constanolactone **33** likely.

The coralline red algae, or Corallinaceae, incorporate calcium carbonate, as do the invertebrate corals, for rigidity and structural strength. These algae, some of which look more like mineral specimens than plants, have proven to be excellent eicosanoid sources. From Brittany waters, Pietra and co-workers reported that a mixture of *Lithothamnion* species yielded a number of novel eicosanoids as ethyl esters.[124] The ethyl esterification is probably an artifact resulting from the ethanol used as a preservative for that collection. The novel hydroxylated compound (**34**) and its ω-6 homolog (**35**) from *Lithothamnion* are unusual in that they lack the conjugated diene characteristic of lipoxygenase activity. However, the isolation of the known eicosanoids 5*S*-, 11*R*-, 12*S*-, and 15*S*-HETE, all as ethyl esters, indicates that *Lithothamnion* possesses multiple lipoxygenase activities as well. The biological characteristics of 5*S*-HETE are similar to 12*S*-HETE, yet further include induction of hexose uptake and calcium flux in mammalian tissues. The immunosuppressor 15*S*-HETE also modulates 5- and 12-lipoxygenases, as well as T-cell immune functions.[125]

Considerably less is known about the oxylipin content of the brown algae. PUFAs from edible *Cladosiphon okamuranus* were shown to exert an allelopathic (growth inhibitory) effect on many microalgal species and macroalgal conchospores.[126] PUFA metabolism in *Notheia anomala*, an epiphyte of another brown alga, *Hormosira banksii*, produces a series of epoxy-, diol-, and tetrahydrofuranyl-lipids which presumably are formed via the decarboxylation and oxygenation of PUFAs.[127] Similar pathways may play a role in the production of the volatile brown algal sex pheromones.[128] Kelps of the family Laminariaceae show promise as a source of new oxylipins. *Ecklonia stolonifera* from Japan produces ecklonialactone B (**36**) and its Δ-6 homolog, ecklonialactone A (**37**), which was a mild feeding deterrent to abalone.[129]

Blue-green algae grow in marine, freshwater, and terrestrial environments. Some of the first examples of oxylipin metabolism in a marine plant were found in *Lyngbya majuscula*, a cyanophyte found in the Caribbean and Pacific. The simple methoxy fatty acid, (**38**), was isolated from the lipid extract of a South Pacific collection.[130] Soon afterwards, it was that discovered that compound **38** was also incorporated into the structure of malyngamide (**39**) a novel amide.[131] Malyngic acid (**40**), a trihydroxy fatty acid, was later isolated from the same alga.[132] In a report on the chemical constituents of a Puerto Rican collection of *L. majuscula*, Gerwick and co-workers discovered cytotoxic and antibacterial activity in two new malyngamides, as well as antimicrobial activity for the methoxy fatty acid **38**.[133] A species of *Oscillatoria* is reported to contain lipoxygenase activity.[134] Comments on lipoxygenase metabolism in freshwater green algae are presented in Chapter III of this thesis.

Oxylipin Metabolism in Terrestrial Plants and Fungi.

As mentioned before, the first lipoxygenase enzyme was isolated from the soybean.[54] Since then, lipoxygenase activity has been found throughout the plant kingdom. Rather than present an exhaustive review of lipoxygenase metabolites from higher plants, examples will be selected which illustrate some of the roles

these substances play in the plants themselves. Recent reviews have been published in this field;[135-137] and so, some of the conclusions made in these articles are summarized in the discussion which follows. The most common PUFAs in terrestrial plants are linoleate (**41**) and α-linolenate (**42**), which are commonly converted by lipoxygenase into either 9- or 13-hydroperoxy intermediates. The regio- and stereo-specificity of the reaction depends on the plant source of the enzyme, pH, and possibly other factors.

The resulting reactive hydroperoxides may be consumed in a variety of fashions. Dehydration may occur to form oxo-acids, such as 9-oxo-(10*E*,12*Z*)-octadecadienoate, which is found in the leaves of *Glechoma hederacea*.[138] The hydroperoxides are also reduced to secondary alcohols in *G. hederacea* and many other plants. In *G. hederacea*, these products are found as free acids and esterified to glycerol-lipids. As in the case of the MGDGs and DGDGs from the red alga *Gracilariopsis*,[113] it is not known if the *Glechoma* lipoxygenase acts on the esterified PUFA, or if the acid is esterified following the lipoxygenase reaction.

In the carnation flower[139] and other plants, lipoxygenase activity has been implicated in the process of senescence, presumably assisting in the degradation of membrane phospholipids. However, in the soybean, it was demonstrated that elevated lipoxygenase activity is not a universal characteristic of senescent tissue.[140] This finding was substantiated in a study with wheat, which concluded that an important function of lipoxygenase may be the consumption of O_2, acting like an antioxidant, and thus enhancing long-term seed viability.[141]

In many plants,[142] including the freshwater green alga *Chlorella pyrenoidosa*,[143] 9- and 13-hydroperoxy fatty acids are cleaved by a hydroperoxide lyase enzyme into aldehyde fragments. Many C_6 aldehydes are volatile and are responsible for producing odors and flavors in food plants. A longer aldehyde resulting from scission at C-13 by hydroperoxide lyase is 12-oxo-(10*Z*)-dodecenoate, trivially named traumatin.[144] Traumatin, produced by plants in response to wounding, also mimics the effects of wounding upon administration.

The production of lipoxygenase metabolites in plants could be a defensive adaptation, as some of the small, volatile aldehydes are antimicrobial. An

antifungal compound from the taro plant *Colocasia antiquorum* has been identified as 9,12,13-trihydroxy-(10*E*)-octadecenoate of undefined stereochemistry at the hydroxyl centers.[145] Such an arrangement could result from the hydrolysis of a hepoxilin A-type intermediate. This antifungal metabolite is produced by the plant in response to infection by the fungus *Ceratocystis fimbriata*.

Jasmonic acid (**43**) is a widely distributed plant growth regulator. This compound, and its volatile methyl ester, which is an important perfume ingredient, inhibit growth and promote senescence,[146] and may also help transduce the stress response within plant tissues.[147] The cyclopentanone arrangement of atoms in jasmonate is reminiscent of the prostaglandins. Jasmonate biosynthesis occurs via an allene oxide pathway[148,149] in a very similar fashion to the one proposed for marine prostanoid biosynthesis in Figure I.4. Recently, allene oxide synthase (dehydrase) from flaxseed was identified as a cytochrome P_{450}.[150] Action of an allene oxide cyclase gives a cyclopentenone product, which, following a series of β-oxidations, yields jasmonate.

Before leaving the higher plants, it should be mentioned that protein kinase C, an enzyme found in both plants and animals,[136] has been implicated in tumor promotion and is stimulated by diacylglycerol lipids. In plants this enzyme may also be intra- or inter-cellularly modulated by eicosanoid metabolites.

Fungi are also a rich source of PUFAs, some of which are then oxidized enzymatically.[151] The purified lipoxygenase of the yeast *Saccharomyces cerevisiae* has similar properties to higher plant lipoxygenases.[152] Hamberg and co-workers have extensively studied the biosynthesis of lipoxygenase products in the primitive fungus *Saprolegnia parasitica*.[153-156] A factor promoting precocious sexual development in the ascomycete *Aspergillus nidulans* was reported as a solvent-extractable entity in the fungal culture medium.[157] The structures of two of the active principles in the medium extract were determined to be the hydroxy-PUFA lactones psiAα (**44**) and psiAβ (**45**).[158] As seen in other bioactive fungal hydroxy fatty acids,[159] the lack of a conjugated diene vicinal to the position of oxidation indicates that true lipoxygenase participation in their biosynthesis is unlikely. Cybaric acid, (**46**), a new metabolite from the chantrelle mushroom *Cantharellus*

cibarus, is formed enzymatically from endogenous lipids in response to wounding of the fruiting body.[160]

In summary, oxylipin metabolism is highly developed in many of the organisms studied thus far. In mammals, eicosanoids and other oxylipins are essential to reproduction, inflammation, enzyme modulation, and the immune response. In the marine invertebrates, analogous pathways are implicated in a variety of functions, including chemical defense and reproduction. It is noteworthy that oxylipin metabolism appears to have been highly conserved evolutionarily. Because no reason for oxylipin metabolism in the marine algae has been conclusively demonstrated conclusively, reproductive and chemical defense motives cannot be ruled out. As in the higher plants, it may be more likely that oxylipin metabolites in the marine algae may serve as growth regulatory hormones, or as primary and/or second messengers in the transduction of stress or wound responses. H^+/K^+ ATPases operate in higher plants,[161,162] so considering the many marine oxylipins which modulate ATPases and other enzymes in vitro, a similar function in seaweeds is also plausible.

Marine Natural Products from the Red Alga *Laurencia*.

The red algal genus *Laurencia* is probably the most chemically productive and best characterized algal genus. Erickson's review on the subject,[163] although dated, is an excellent compilation of *Laurencia* chemistry. The main chemical structure classes found in *Laurencia* are sesquiterpenoids, diterpenoids, and C-15 acetogenins. Many of these metabolites are halogenated, presumably through the action of haloperoxidase enzymes. A number of *Laurencia* metabolites exhibit cytotoxicity or antimicrobial activity. It has been suggested that the halogenated *Laurencia* metabolites may provide the alga with a selective environmental advantage, for example, by inhibiting overgrowth of the alga by microflora or by deterring predation by herbivorous grazers.[164]

Because the genus *Laurencia* is a morphologically variable, taxonomically difficult group, the use of its halogenated metabolites as chemotaxonomic markers

has been suggested.[165] Terete forms of *Laurencia*, in which the thallus is cylindrical, contain a crystalline structure, the *corps en cerise*. This structure is inside the trichoblast and outer cortical cells, and is the site of halogenated metabolite storage. The non-terete compressed (flattened) forms of the genus, exemplified by *L. chilensis* and *L. spectabilis*, contain neither *corps en cerise* nor halogenated organic molecules.[166]

Two new chamigrenes, obtusadiene **47** and isoobtusadiene **48**, from tropical *L. obtusa*, are presented as examples of terete *Laurencia* sesquiterpenoid metabolism.[167] These two novel compounds lacked significant antimicrobial or cytotoxic activity, although related *Laurencia* sesquiterpenes are known to be cytotoxic.[79] With the exception of one other algal genus, bromo-diterpenes are only found in *Laurencia*, as exemplified by concinndiol (**49**) from *L. concinna*.[168] Diterpenes from *Laurencia* are not nearly as commonplace as sesquiterpenes, while monoterpene metabolism appears to be entirely absent in *Laurencia*.

The C_{15} acetogenins are a *Laurencia* structure class which appear to be derived from PUFA metabolism. Erickson's[163] and Fenical's[164] reviews list scores of cyclic and acyclic acetogenins, many of which are halogenated. The acetylenic laurencenynes (**50-53**) from *L. okamurai* are evidence for PUFA biogenesis of the C_{15} acetogenins.[169] The laurencenynes are found in both the ω-3 and ω-6 configurations, with olefins and bis-allylic methylenes properly situated in a PUFA-like carbon chain. A different pathway of *Laurencia* PUFA metabolism is discussed in Chapter II of this thesis.

A minor *Laurencia* structure class which also contains a linear arrangement of carbon atoms, in this case, linear C_5 units, is represented by the racemic crystalline chilenone A (**54**)[170] Chilenone A was recovered from dried *L. chilensis* which was Soxhlet-extracted with acetone. Chapter IV of this thesis further explores the distribution of this uncommon chemical structure class from *Laurencia*, and examines its unique chemical reactivity.

Final examples of the diversity of *Laurencia* secondary metabolism comes from *L. brongniartii*, a flattened *Laurencia* species. Bromoindoles **55-58** were isolated from a Caribbean collection,[171] from which only compound **57** exhibited

antimicrobial and cytotoxic activity. The discovery of these compounds necessitates revision of the chemotaxonomic hypothesis, put forth by Young et al.,[166] that flattened *Laurencia*, due to their apparent lack of *corps en cerise*, do not contain halogenated organics. Erickson's review[163] reports a unpublished finding by H.H. Sun that Taiwanese *L. brongniartii* contains methylthiobromoindoles **59-62**. Methylthiobromoindoles have been proposed as precursors of the mollusc-derived pigment Tyrian purple (**1**).[172]

Indole-Containing Marine Natural Products.

The field of marine indoles was comprehensively reviewed by Christophersen[173] and also by Fenical within the category of marine alkaloids.[174] Although approximately one hundred indole compounds have been isolated from marine sources, this section will focus on structurally simple examples, especially those which display biological activity. Among the common amino acids, the indole functionality is unique to tryptophan. Many important plant and animal hormones are derived from tryptophan. For example, 5-hydroxytryptamine (serotonin, **63**) is a neurotransmitter in the human brain.

In plants, indole acetic acid (IAA, **64**) is a common auxin, or growth stimulating substance. In some parts of the world, seaweeds are applied to fields as fertilizer, in part because of their auxin content.[175] Fries demonstrated improved growth in the cultured marine algae *Ascophyllum nodosum*[176] and *Fucus spiralis*[177] when IAA was added to their culture media, implying endogenous production and reception of indole auxins in the algae. As mentioned in the previous section, the red algal genus *Laurencia* is a source of novel halogenated indoles. The red alga *Rhodophyllis membranacea* was shown to produce a complex mixture of antifungal polyhalogenated indoles, some containing chlorine as well as bromine.[178] In the dinoflagellate *Gonyaulax polyedra*, photoperiodism is regulated by 5-methoxytryptamine and by melatonin, (**65**), a known mammalian photoregulatory hormone.[179] This latter finding suggests a common biochemical pathway for the synchronization of light/dark cycles in plants and animals.

The marine invertebrates are better known than marine plants as producers of novel indoles. Tryptamine and tryptamine metabolites secreted by sea anemones elicit stereotypical behavior patterns in fish which live in symbiosis with the anemones.[180] The anemone fish live in close proximity to the poisonous anemone tentacles, but are immune to their poisonous sting, thus deriving protection from predatory fish.

The New Zealand ascidian *Ritterella sigillinoides* produces the indole-containing eudistomin K sulfoxide **66**, which displays *in vitro* activity against *Herpes simplex* Type I and *Polio* Type I viruses.[181] The Okinawan sponge *Hyrtios erecta* produces cytotoxic 5-hydroxyindole-3-carboxaldehyde along with the novel hyrtiosin A (**67**) and the apparent dimer hyrtiosin B (**68**).[182] Marine animals, especially sponges, are known to harbor algal and bacterial organisms within their tissues. It is important to consider the ultimate origin of marine invertebrate indoles, as indole-3-carboxaldehydes have been isolated from a marine pseudomonad bacterium,[183] and are common in the marine algae.[184-186]

Summary.

The marine environment is a vast resource of novel compounds which possess a diversity of ecological, physiological, and pharmacological functions. Scores of academic and industrial research groups around the world are currently exploring various facets of this exciting and multidisciplinary field. However, the true ecological and physiological significance of many marine natural products, including the ones described in the following chapters, remains to be discovered. Very few novel compounds ever make it to market, but certainly some of today's and tomorrow's marine natural products will find utility as structural templates for the design of new pharmaceuticals and agrochemicals, as well as probes for unravelling the intricacies of the human organism.

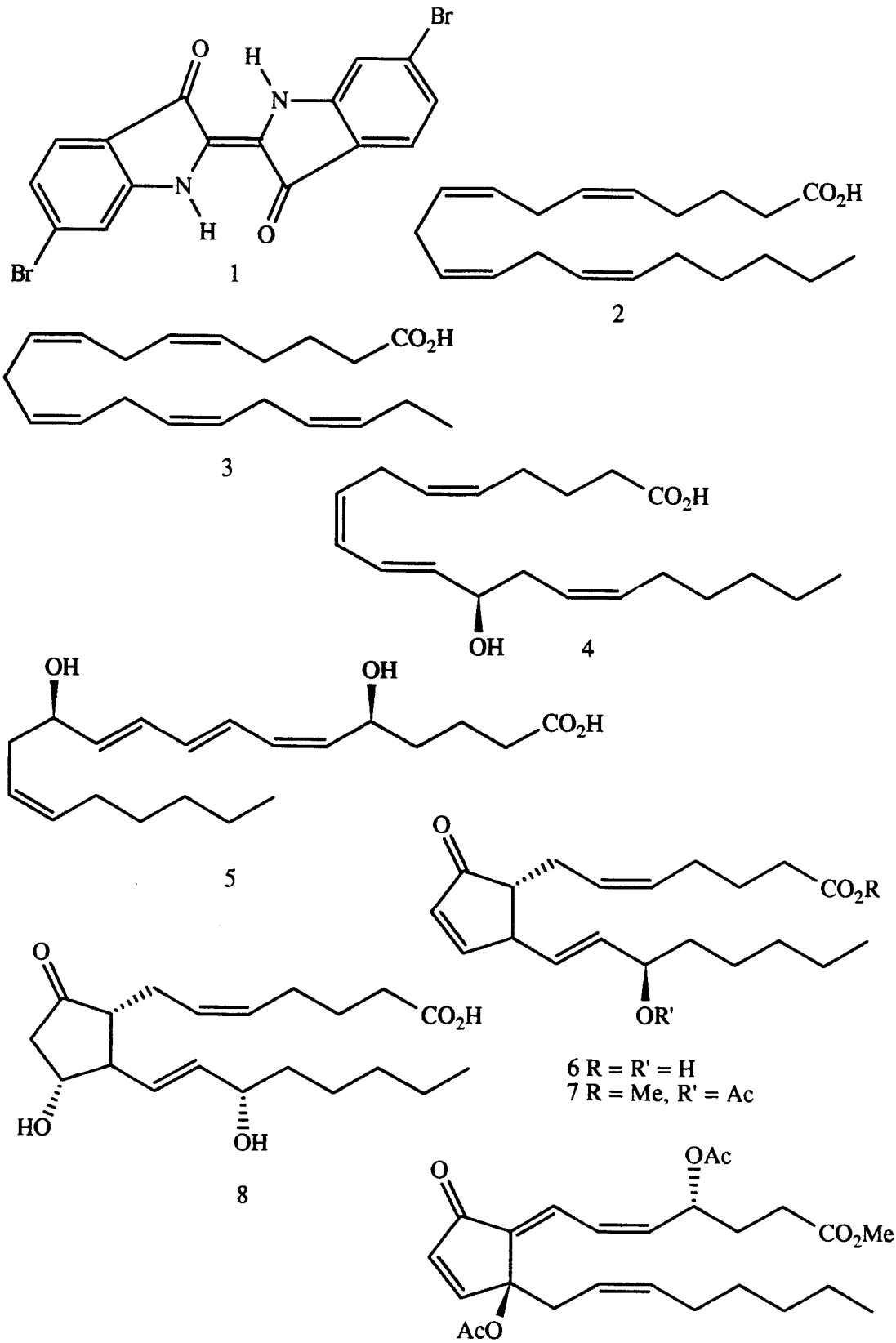
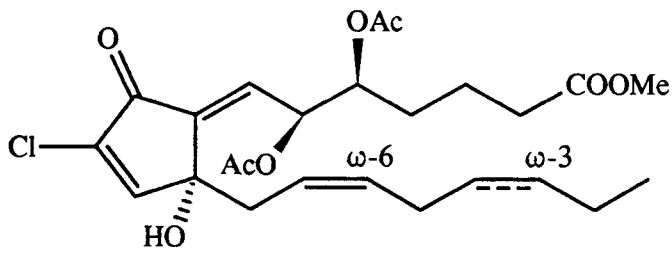
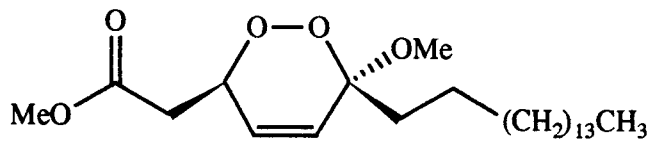


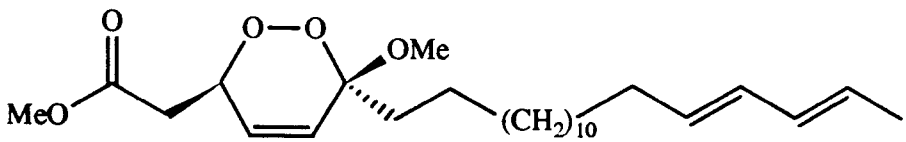
Figure I.1. Chemical structures for Chapter I.



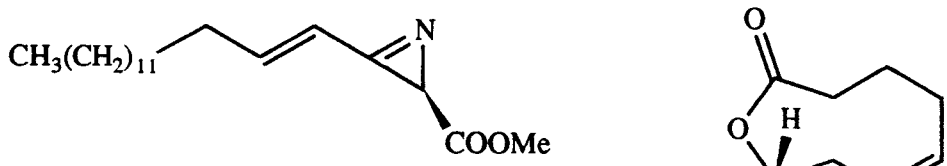
10 ω-3
11 ω-6



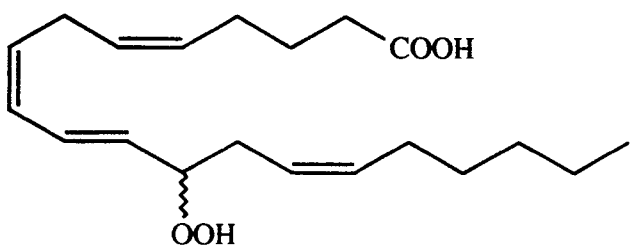
12



13

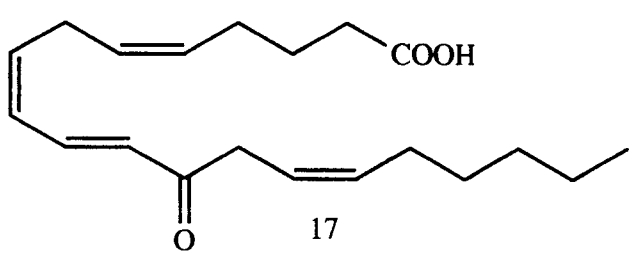


14



15

16



17

Figure I.1 (Continued)

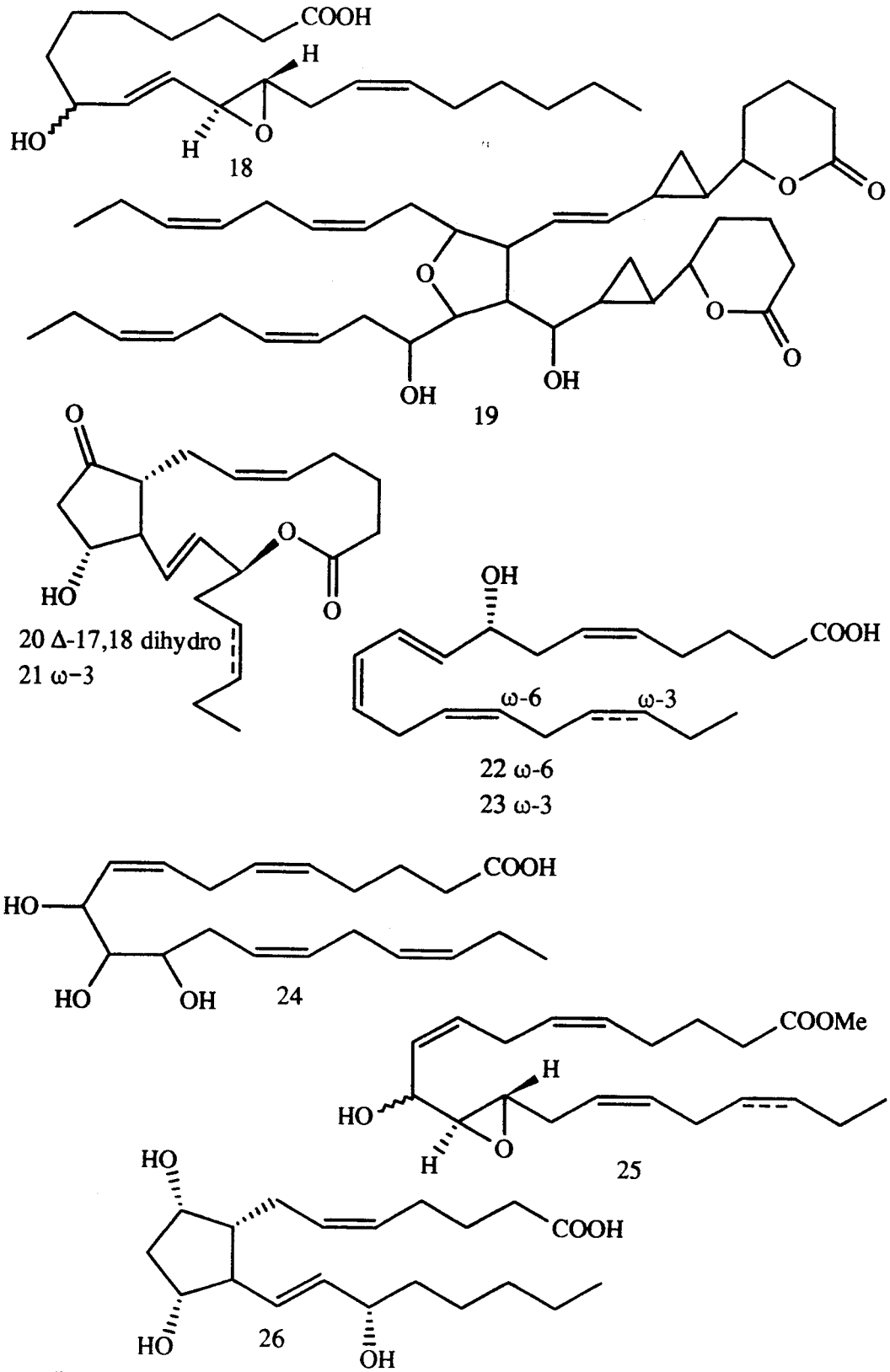


Figure I.1 (Continued)

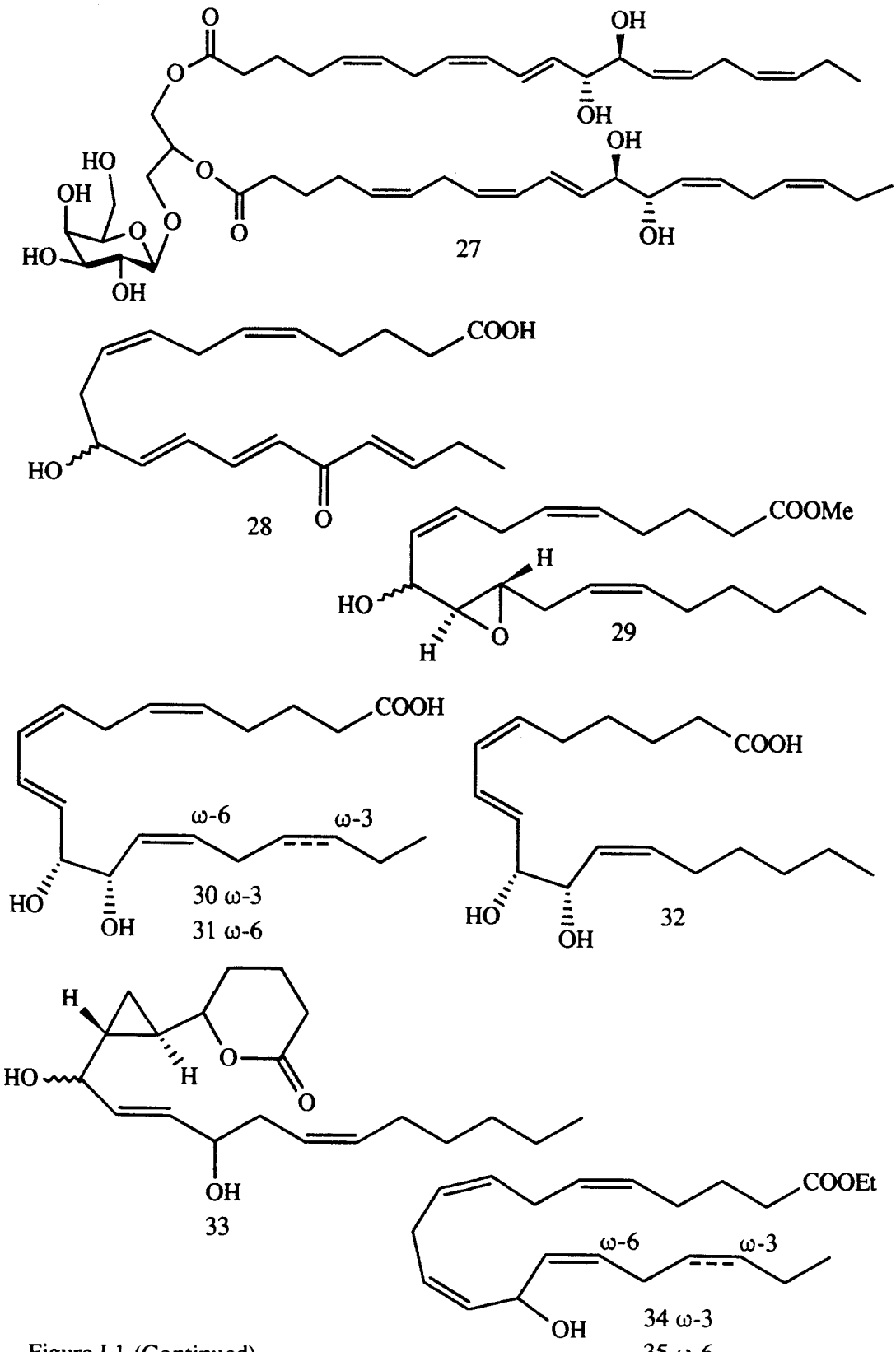


Figure I.1 (Continued)

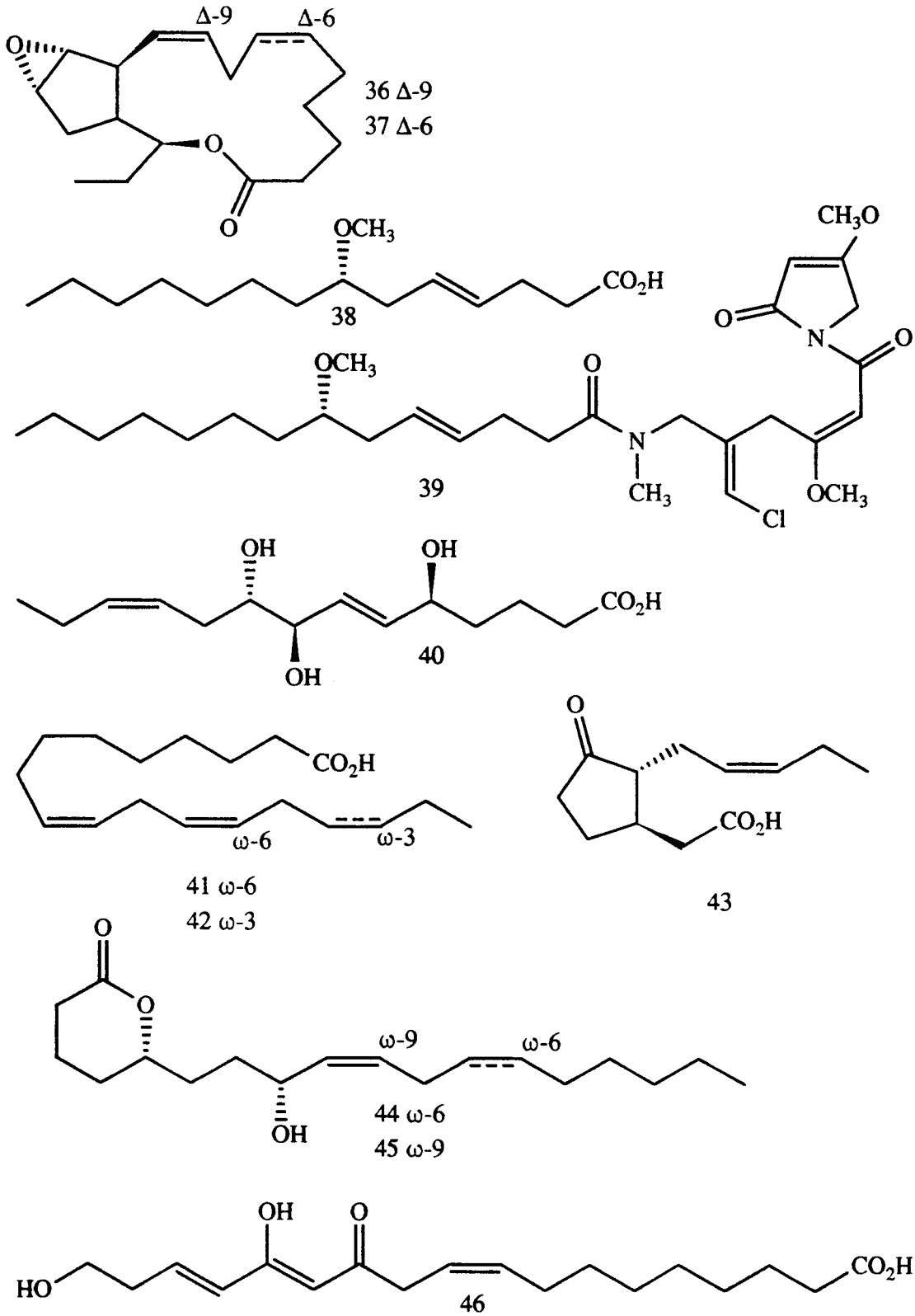
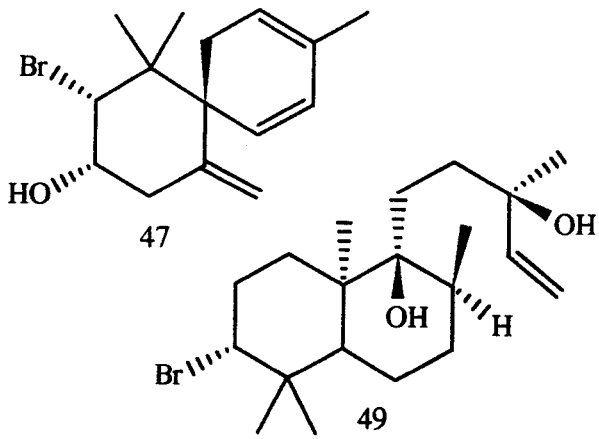
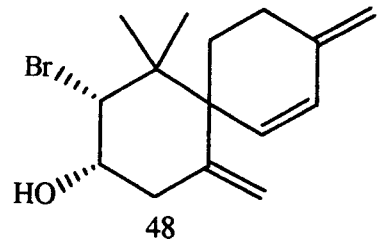
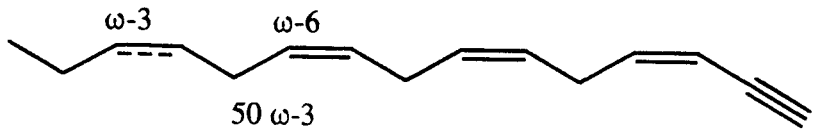


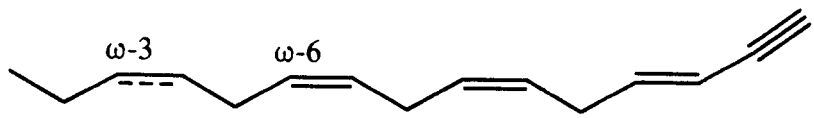
Figure I.1 (Continued)



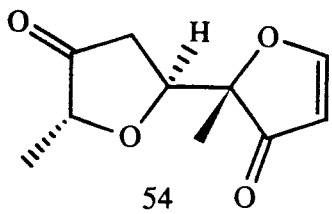


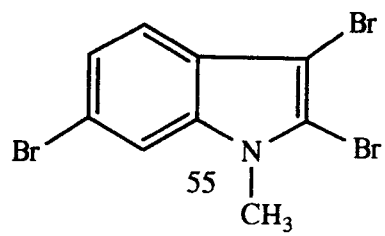


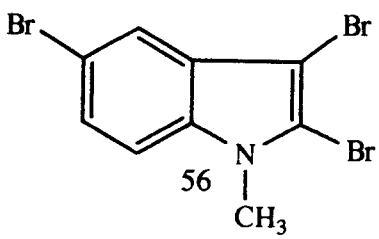
50 ω-3
51 ω-6

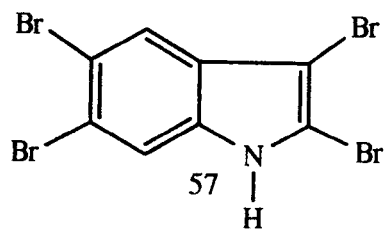


52 ω-3
53 ω-6









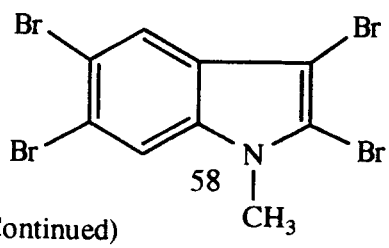
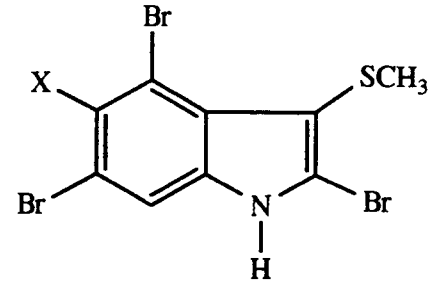
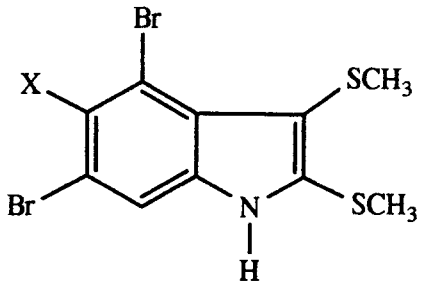


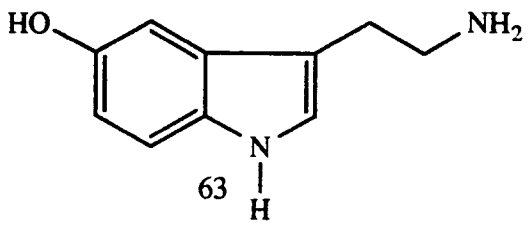
Figure I.1 (Continued)



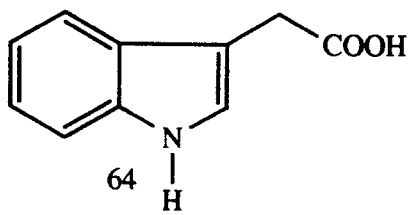
59 X = H
60 X = BR



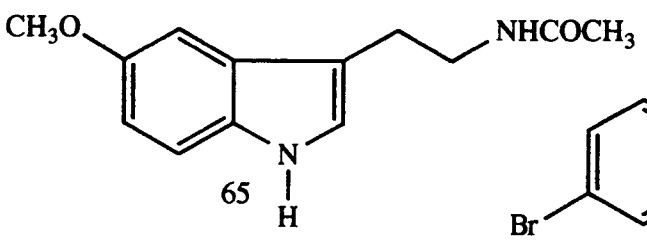
61 X = H
62 X = BR



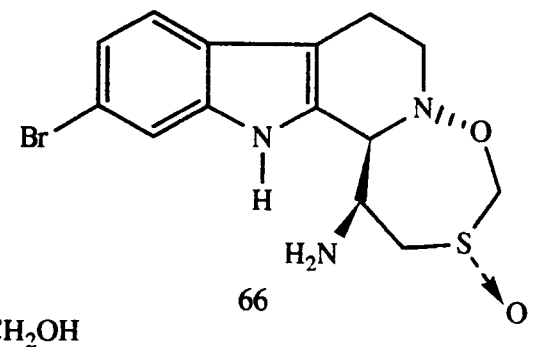
63



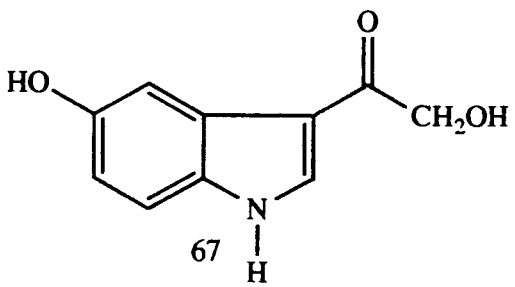
64



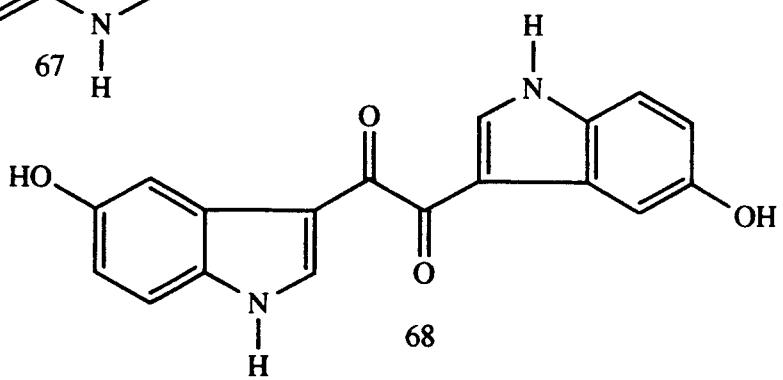
65



66



67



68

Figure I.1 (Continued)

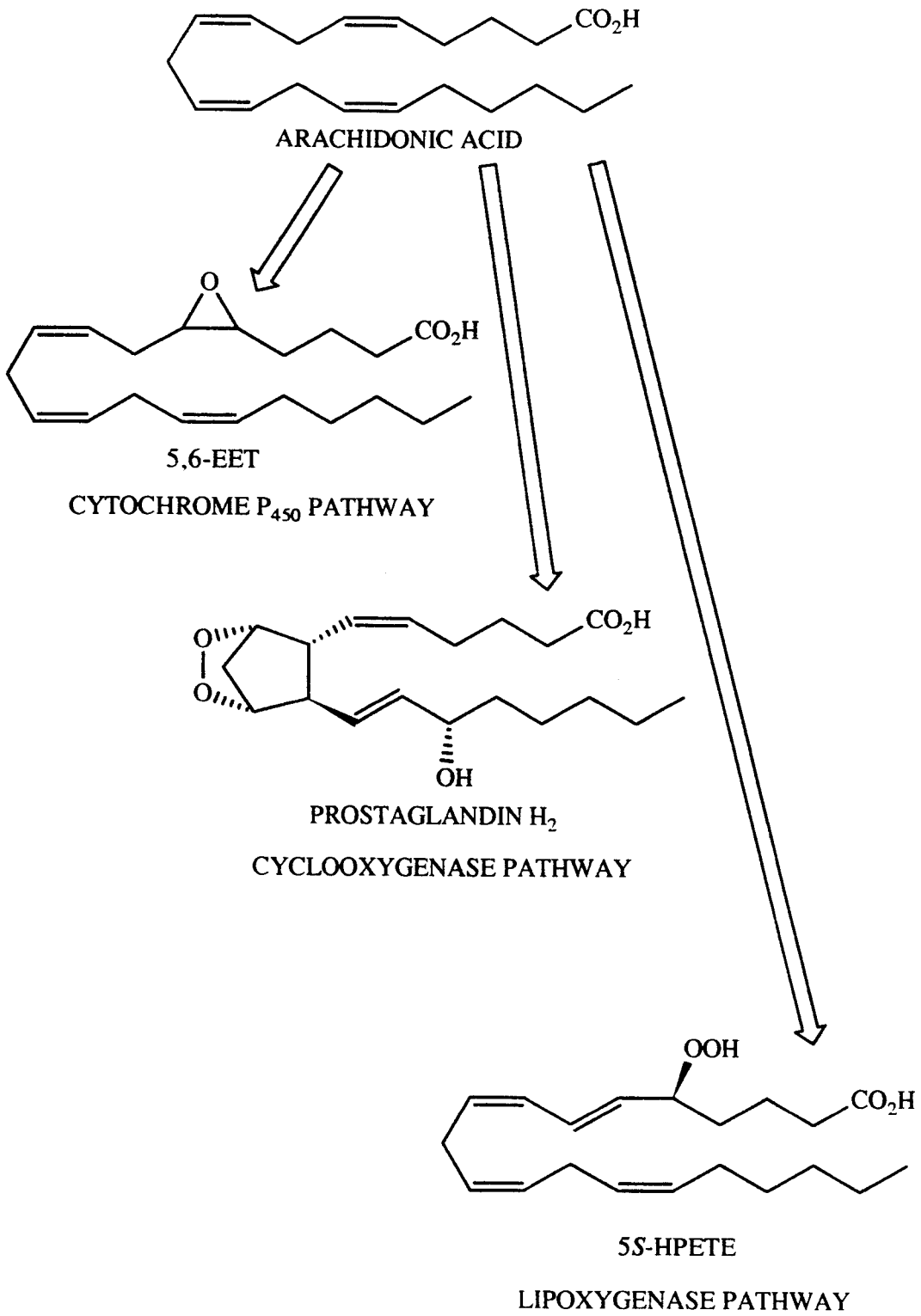


Figure I.2. Mammalian pathways of eicosanoid metabolism.

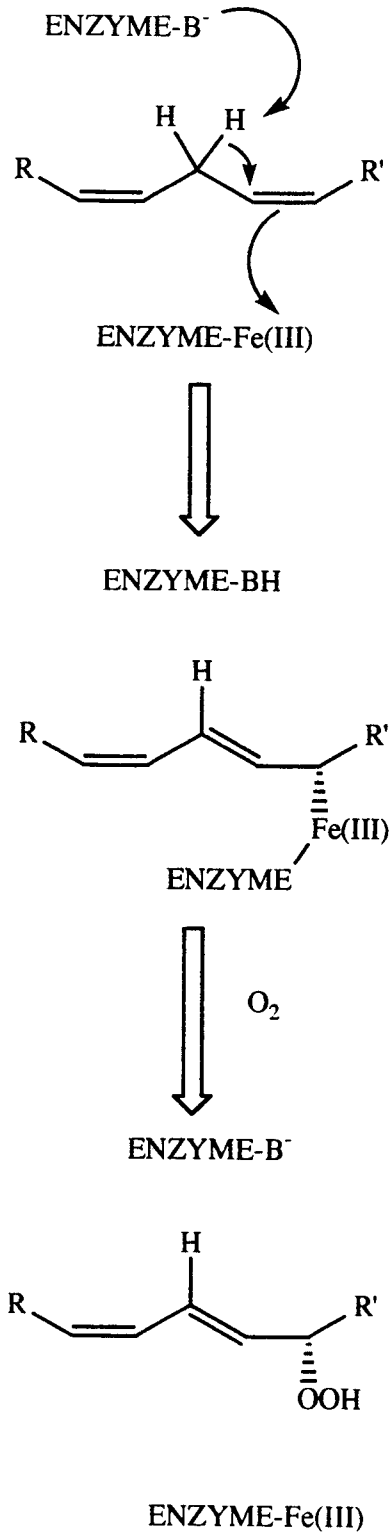
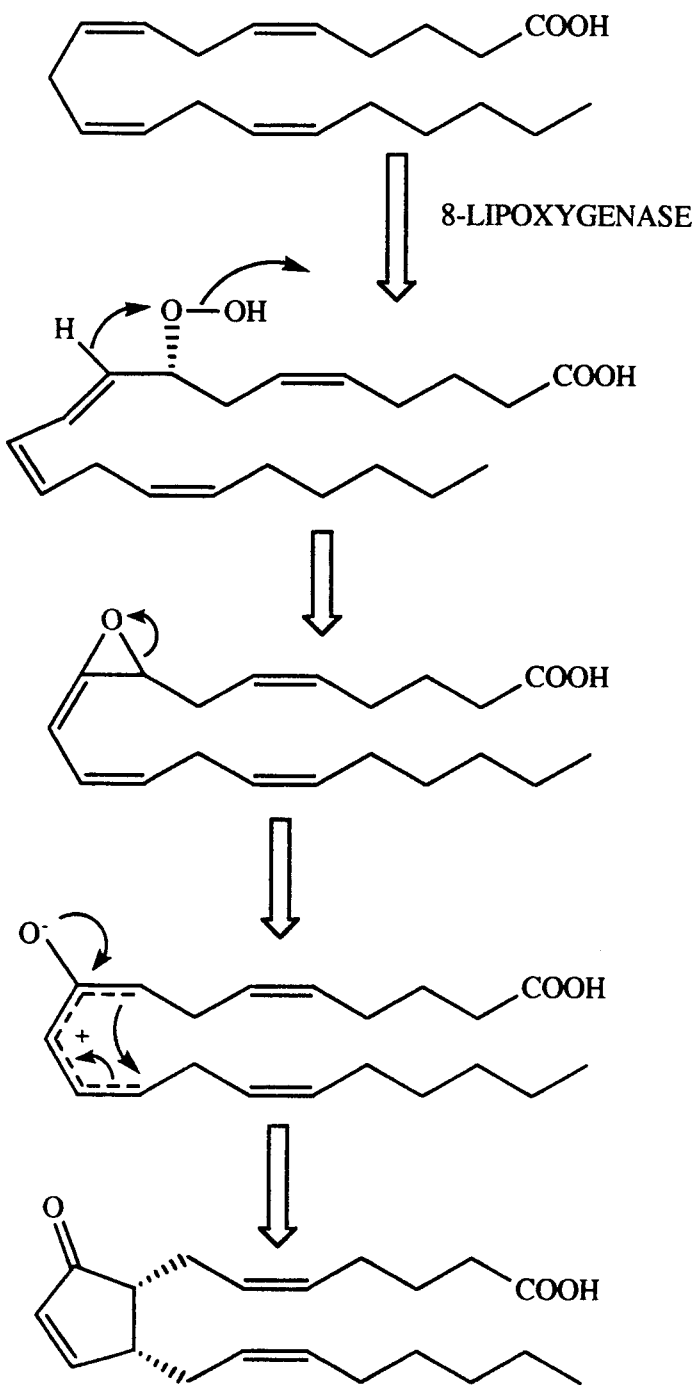


Figure I.3. Proposed mechanism of the lipoxygenase reaction.



PRECLAVULONE A

Figure I.4. Allene oxide pathway to marine prostanoids.

CHAPTER II

EICOSANOID NATURAL PRODUCTS FROM THE TROPICAL RED ALGA *MURRAYELLA PERICLADOS* (C. Ag.) SCHMITZ

ABSTRACT

The tropical red marine alga *Murrayella periclados* is a rich source of a number of eicosanoids which are biologically active in mammalian systems. This includes 12*S*-HEPE and 12*S*-HETE, (6*E*)-LTB_4, and two diastereomers each of hepoxilin B_3 and B_4, all of which were identified by spectrochemical methods. Comparison of data for 12-HEPE with that for a reported hydroxyeicosapentaenoic acid from the related seaweed *Laurencia hybrida* revised the *L. hybrida* structure to 12-HEPE, which had been hypothesized as an intermediate in the biosynthesis of the unusual *L. hybrida* eicosanoid hybridalactone. New enzyme inhibitory activities are reported for some of these algal eicosanoids.

INTRODUCTION

Prior to the present investigation, the natural products chemistry of the red alga *Murrayella periclados* (C.Ag.) Schmitz had not been studied. *M. periclados* belongs to the family Rhodomelaceae, order Ceramiales, class Florideophyceae of the division Rhodophyta. Characteristic of mangrove thickets and other quiet, shallow, saltwater locations throughout the Caribbean,[187] *M. periclados* possesses a typical *Polysiphonia*-type life history, with separate gametophytic and tetrasporophytic plants.[188] The gametophytes are both monoecious, bearing both male and female sex functions on the same plant, as well as dioecious, in which case a plant is either entirely male or entirely female. *M. periclados* is quite common to the world's tropical regions, with published reports ranging from the Yucatan Peninsula of Mexico[189] to the Mozambique border region of South Africa.[190] Additionally, collections have been made in Australia and the Philippines.[191]

Preliminary investigations had hinted at the biological activity of *Murrayella* extracts. In a survey of the antibiotic activity of some Puerto Rican marine algae, aqueous slurries of *M. periclados* were found to exhibit antimicrobial activity against *Staphylococcus aureus, Escherichia coli*, and *Mycobacterium smegmatis*.[192] In a more recent study of lipid soluble algal extracts from the same region[193], *M. periclados* was characterized by the sensitivity disk method as devoid of antimicrobial activity. However, lipid extracts of subsequent collections from Isla Guayacan, near La Parguera, Puerto Rico, showed sporadic activity against *S. aureus* and *Candida albicans*, and contained interesting UV-active metabolites by TLC analysis, prompting this investigation.

Our interest in *Murrayella* led us to an early report[194] of antimicrobial activity in a British red alga from the same taxonomic family, *Laurencia hybrida*. This report was followed by the isolation of antimicrobial components from *L. hybrida* which clearly derived from polyunsaturated fatty acid metabolism.[195] Soon afterwards, workers in our laboratory[118] discovered that crude organic extracts of *Ptilota filicina*, also a member of the Rhodomelaceae, were antimicrobial, and

indeed contained a novel eicosanoid which was at least partially responsible for the antimicrobial activity. This observed pattern of antimicrobial activity corresponding to the presence of eicosanoid chemistry is not restricted to the family Rhodomelaceae or the order Ceramiales. An unrelated Oregon red alga, *Farlowia mollis*, displays the same phenomenon.[121] Widespread antimicrobial activity in algal[196] and other marine lipid extracts[13] is likely due to the detergent action of algal fatty acids on the cell walls of micro-organisms employed in these biological assays.

Through the use of a bio-autographic TLC technique, one of two UV-active antimicrobial compounds from *L. hybrida* was identified as the unusual 9-hydroxy-(2*Z*,5*Z*,7*E*,11*Z*,14*Z*)-eicosapentaenoic acid (**6**).[195] Several aspects of the structure elucidation presented for this compound were questionable, even according to the author, Higgs, who noted no deshielding of the vinyl protons in the α,β-unsaturated ester of his proposed structure. Moreover, the placement of a double bond at the Δ-2 position of the fatty acid chain is inconsistent with all accepted concepts of polyunsaturated fatty acid biosynthesis.[197] Also debatable was the assignment of chemical shifts δ2.06 and δ2.32 to the ω-2 and ω-3 position methylenes, respectively, of the purported ω-6 fatty acid. These latter two chemical shifts are too far downfield to be normal aliphatic, alkyl chain proton resonances.

In the course of our structure elucidation studies on the *Murrayella* eicosanoids, data was collected which allowed the reassignment of the structure of the unusual eicosanoid from *L. hybrida* to (12*S*)-hydroxyeicosapentaenoate (12*S*-HEPE). This was additional evidence for the intermediacy of 12*S*-HEPE in the biosynthesis of the unusual cyclized eicosanoid, hybridalactone[198,199], from the same alga. In summary, a number of known eicosanoids, some of which are important mammalian immunoregulators, were isolated from *Murrayella periclados*. Many of them were purified in greater amounts than had ever been reported previously. New enzyme inhibition data is reported for some of these algal-derived eicosanoids.

RESULTS AND DISCUSSION

Summer collections of *Murrayella periclados* from Isla Guayacan, Puerto Rico, in 1984 and 1985 were preserved in isopropanol (iPrOH) and transported to Oregon for further analysis. The crude organic extract displayed a prominent low R_f (relative to the solvent front), tailing, UV-active region on TLC. Vacuum chromatography (VC)[200-202] of a large collection resulted in a series of crude lipid fractions of increasingly polar content. Those which eluted from the column in 30-40% ethyl acetate (EtOAc) in 2,2,4-trimethylpentane (TMP) were enriched in UV-active compounds. These compounds were methylated with ethereal diazomethane (CH_2N_2), thus improving their chromatographic behavior.

Unoxidized fatty acid methyl esters.

Following methylation of the above compounds, a second VC, followed by HPLC of the UV-active fractions, resulted in the isolation of three eicosanoid compounds. The first of these to elute was a polyunsaturated fatty acid methyl ester. All of the methylenes in the proton nuclear magnetic resonance (^{1}H NMR) spectrum were either bis-allylic (8H, δ2.85), vicinal to the ester (2H, δ2.10), allylic (4H, δ2.10) or borne on C-3 (2H, δ1.63). The low resolution electron impact mass spectrum (LR EIMS) was a very close match to that published by Alexander and co-workers for methyl arachidonate.[203] However, methyl arachidonate would have displayed aliphatic signals for the protons at C-17,18, and 19, which were not observed in the NMR spectrum. The molecular ion $[M]^+$ of methyl arachidonate appears in the published mass spectrum at *m/z* 318. This same peak was observed in our mass spectrum as well. However, $[M]^+$ of methyl eicosapentaenoate (**1**) was observed at *m/z* 316, corresponding to the molecular formula of $C_{21}H_{32}O_2$. One possible explanation for our *m/z* 318 peak is hydrogenation of a double bond during MS analysis, a phenomenon known to occur during chemical ionization (CI) MS of polyunsaturated fatty acid methyl esters.[204] A more likely explanation is that the sample of compound **1** was

contaminated with a small amount of methyl arachidonate which was not detected in the ^{1}H NMR analysis.

Eicosapentaenoic acid (EPA), a natural product of *M. periclados*, is a common algal fatty acid,[35] and is important in the human diet.[30] Fish oils contain EPA as a major component, and have been shown to lessen some of the symptoms or incidence of diseases such as psoriasis,[31] arthritis,[32] and coronary heart disease when administered to humans.[33] Part of the mechanism of action of these effects may result from the substitution of EPA for arachidonic acid (AA) as a substrate for pro-inflammatory cyclooxygenase and lipoxygenase enzyme pathways. It is not fully known whether the effect is mainly due to EPA's inhibition of cyclooxygenase or lipoxygenase activity, or if the resulting metabolites of EPA are less inflammatory than those of AA.[205] The structure of EPA methyl ester (**1**) and other compounds isolated from *M. periclados* are depicted in Figure II.1.

The next compound eluting from the column during this preparative HPLC work was UV-active on TLC. Comparison of its IR and ^{1}H NMR spectra to previously published data from our laboratory[206] identified it as methyl (5*Z*,7*E*,9*E*,14*Z*,17*Z*)-eicosapentaenoate (**2**), which was first isolated from the related Oregon red alga *Ptilota filicina*, and subsequently discovered in the red alga *Farlowia mollis*.[121] In *Ptilota*, *Farlowia*, and *Murrayella*, TLC analysis showed that compound **2**, although isolated as the methyl ester, was in the free acid form as the natural product.

The last compound resulting from this HPLC run had UV and ^{1}H NMR spectra which matched those reported for methyl (5*E*,7*E*,9*E*,14*Z*,17*Z*)-eicosapentaenoate (**3**), also isolated from *P. filicina*. It was observed that during storage, isomer **2** would slowly rearrange to isomer **3**. The all *E* arrangement is sterically less crowded and thermodynamically more stable. Although Lopez and Gerwick first speculated that both isomers **2** and **3** are true natural products of *P. filicina*, it seems likely that the *Z,E,E*-isomer **2** (as the free acid) is formed first and is the only true natural product of the two isomers found in these three red algae (*Murrayella, Ptilota,* and *Farlowia*).[53]

During the course of these purifications, a UV-inactive oil which charred blue on TLC upon acidification, was characterized by ^1H-^1H COSY correlation NMR to be a triglyceride of extremely similar structure to one isolated from *Argemone mexicana* (Papaveraceae).[207] This terrestrial plant produces *sn*-glycerol-1-eicosa-9,12-dienoate-2-palmitate-3-linoleate, which was shown to be toxic to the nematode *Meloidogyne incognita*. Due to a lack of material, the unequivocal structure elucidation of the algal triglyceride was not pursued.

Monohydroxy fatty acids.

The more polar fractions (50-100% EtOAc) from the original VC contained a distinctive UV-active region which tailed on TLC, and was reactive to both CH_2N_2 and acetic anhydride/pyridine, indicating a carboxylic acid and an unhindered hydroxyl group, respectively. Because of its improved chromatographic properties, the acetate, methyl ester derivative (**4**) was produced on a preparative scale and isolated by VC, preparative thick layer (pTLC) and HPLC. For ^1H and ^{13}C NMR data, see Table II.1.

Integration of the ^1H NMR spectrum and examination of the ^{13}C NMR data showed that compound **4** contained five olefins; therefore, it was quite likely a metabolite of EPA. The UV-spectrum displayed an absorbance maximum at 238 nm, indicative of a conjugated diene system,[106] a conclusion which was substantiated by the COSY correlation data. Coupling constant analysis predicted a *cis-trans* diene system. A ^1H-^{13}C HETCOR experiment allowed the assignment of proton resonances to their respective carbons; for example, placing the α-acetoxyl proton resonance in the overlapped multiplet region at δ5.51 on a carbon resonance at δ74.03.

Though partial structures,[208] including the Δ-5, ω-3 nature of the molecule, could be generated from the data at this point, the overlap in the olefinic region of the ^1H NMR prevented the establishment of connectivity and assignment of position of oxidation on the fatty acid chain. Pursuing a different line of experimentation, the oxidized fatty acid metabolite was prepared for mass

spectrometry. A portion of the underivatized material was treated with CH_2N_2 and then purified by VC to yield the methyl ester **5** (^{1}H NMR, Table II.1). The trimethylsilyl (TMS) ether derivative of methyl ester **5** was then analyzed by gas chromatography-mass spectrometry (GCMS). Chemical ionization (CI) GCMS of this derivative detected $[M + H]^+$ *m/z* 405, thus establishing a molecular formula of $C_{20}H_{30}O_3$ for the natural product. The EI mass spectrum of this derivative displayed a characteristic α-cleavage at *m/z* 295, matching published spectra,[106,209] thereby defining the position of oxidation on the fatty acid chain as C-12. Therefore, compound **5** was identified as methyl (12*S*)-hydroxyeicosapentaenoate (12*S*-HEPE methyl ester).

Once the position of oxidation and carbon chain length had been established, NMR assignments for structures **4** and **5** could be made in Table II.1. A favorable comparison of the optical rotation of methyl ester **5**, $[\alpha]_D$ +8° (*c* 0.9, acetone) with that of methyl (12*S*)-hydroxyeicosatetraenoate (12*S*-HETE methyl ester),[210] $[\alpha]_D$ +13° (*c* 1.5, acetone), established the absolute stereochemistry at C-12 of compound **5** to be *S*. These differences in magnitudes of optical rotation were at first ascribed to the possibility of partial racemization of our isolate.

However, subsequent analysis of methyl ester **5** on a dinitrobenzoylphenylglycine (DNBPG) HPLC column, in a duplication of the method of Kuhn et al.,[211] showed a single peak. Had there been any contaminating *R*-enantiomer in our sample, it should have resolved as a separate peak on the chromatogram.[106,211] In all likelihood, the disparity between our optical rotation and the literature value results from any combination of the following factors: a weighing error, a temperature or instrumentation difference between the two laboratories, or an inherent difference between 12*S*-HEPE and 12*S*-HETE methyl esters, the two compounds under comparison.

Consideration of the polyunsaturated fatty acid derived metabolites previously reported from red algae prompted us to analyze the data Higgs reported for the unusual hydroxyeicosapentaenoic acid isolated as the methyl ester (**6**) from *Laurencia hybrida*.[195] The NMR spectra of compounds **5** and **6** were essentially identical (Table II.2), as were the UV and IR spectra, and aspects of

the mass spectra. Probably due to printer's error, both positive and negative optical rotations were reported for Higgs' proposed structure; however, we measured an optical rotation for **5**, $[\alpha]_D$ +4° (*c* 0.65, MeOH) which was of similar magnitude to that reported for **6** using the same solvent.

In retrospect, structure **5** is much better supported by Higgs' data and resolves his troublesome assignments, mentioned in the introduction of this chapter, for the α,β unsaturated ester protons and the ω-2 and ω-3 methylenes. Moreover, Corey proposed that 12*S*-HEPE was an intermediate in the biosynthesis of hybridalactone[198], an unusual cyclic eicosanoid which Higgs had also isolated and identified from *L. hybrida*.[199] Higgs' structure **6** is therefore revised to 12*S*-HEPE methyl ester (**5**). A summary of eicosanoid biosynthesis in *L. hybrida* is presented in Figure II.2. In it, we depict the formation of 12-oxo-(5*Z*,8*E*,10*E*)-dodecatrienoic acid, which Higgs also isolated from *L. hybrida* as the methyl ester.[195] This *Laurencia* metabolite can be envisioned as arising from 12-lipoxygenase acting on EPA to form the 12-hydroperoxide intermediate, which could then be cleaved by hydroperoxide lyase.[212] Hydroperoxide lyase enzyme activity following lipoxygenation is a widespread phenomenon throughout the terrestrial plant kingdom,[135,142] and has been detected in a freshwater green alga as well.[143]

Alternatively, peroxidase activity may reduce the hydroperoxide intermediate to 12*S*-HEPE (**7**) as shown. A third alternative is for electron flow to occur as indicated by the solid arrows in figure II.2. The migration of the C-14,15 olefin π-electrons to C-10 results in the formation of a cyclopentane ring with an 11,12-epoxide with loss of water as shown. Multiple rearrangements of the resulting carbocation were proposed, along with cyclopropyl formation and lactonization to give the hybridalactone structure originally reported by Higgs from *L. hybrida*.[198,199]

Because of the current medical interest in the biological activities of eicosanoids,[61,213] we endeavored to purify the underivatized natural product 12*S*-HEPE (**7**) for biological evaluation. The TLC separation method of Boukhchache and Lagarde[214] was adapted for pTLC of crude fractions in order to recover UV-

active metabolites. This process yielded approximately 70% pure compound **7**. It was this impure material on which we based the ^{1}H NMR spectrum and the neutrophil degranulation data in our original report.[208] Subsequently, we discovered that we could purify relatively large amounts (>1 mg/day) of 12*S*-HEPE by adapting the HPLC system of Cunningham et al.[215] The 12*S*-HEPE ^{1}H NMR spectrum has been updated with data obtained using purified compound **7** (Table II.1); however, the neutrophil degranulation results must still be regarded as preliminary. At current market prices of ca. $3,000/mg (Cayman Chemical Co.), it seemed prudent to attempt to patent the algal source and purification procedure for 12*S*-HEPE. On March 7, 1989, we were awarded U.S. Patent #4,810,424.

During the HPLC purification of natural product **7**, a less polar UV-active substance (**8**) was recovered. Its ^{1}H NMR characteristics were very similar to 12*S*-HEPE (**7**); however, it displayed only one bis-allylic methylene resonance, as well as additional aliphatic methylene signals not seen in the spectrum of free acid **7**. At once we suspected that the compound was 12*S*-HETE, (**8**), which had previously been isolated in our laboratory from the tropical red alga *Platysiphonia miniata* as the semi-synthetic acetate, methyl ester derivative.[119] The optical rotation and ^{1}H NMR spectrum of free acid **8** were comparable to literature values reported for 12-HETE methyl ester.[210] Our conclusion was subsequently proven upon methylation and silylation of **8**, which on GCMS displayed the same characteristic spectrum as originally reported for this derivative by Hamberg and Samuelsson.[216]

We noticed in ^{1}H NMR spectra that the bis-allylic methylene protons at C-7 in both natural products **7** and **8** are rendered diastereotopic. Though C-7 is not vicinal to an asymmetric center (C-6 and C-8 are olefins), diastereotopic methylenes are known to occur in cyclic systems. Such a situation might result from a macrocycle formed by hydrogen bonding of the C-12 hydroxyl proton to the carboxyl terminus (see Figure II.3). Although we attempted a two-dimensional nuclear Overhauser effect correlation experiment (nOeSY) to detect through-space interactions across the ring, no meaningful data resulted from this

effort. Prior to running this experiment, a ^1H-^1H COSY spectrum of free acid **7** was acquired, and is depicted in Figure II.4. In this spectrum it is clear that the bis-allylic methylene at C-16 is not diastereotopic.

A dihydroxy fatty acid metabolite.

A smaller collection of *M. periclados* was collected in Fall, 1984 near La Parguera, Puerto Rico. Vacuum chromatography of the crude extract yielded a series of crude fractions. The fraction eluting in 40% EtOAc contained a polar UV-active material which acetylated but did not methylate. Because a previous collection of this alga contained an incompletely characterized and unstable leukotriene-like compound (W.H. Gerwick and A.B. Alvarado, personal communication), the entire fraction was acetylated overnight in an attempt to stabilize any allylic hydroxyls. A second VC followed by repetitive HPLC resulted in the isolation of 2 mg of the compound of interest (**9**). The UV spectrum showed a triad of maxima at 260, 270, and 281 nm in the characteristic pattern of a conjugated triene system.[217] The IR spectrum displayed typical aliphatic and olefinic absorptions as well as a strong ester carbonyl. In the high resolution mass spectrum, we observed $[M]^+$ *m/z* 448.2825, which gives a molecular formula for the derivative of $C_{26}H_{40}O_6$, and results in seven degrees of unsaturation.

The one- and two-dimensional ^1H NMR spectra (COSY, Figure II.5) of compound **9** were quite complex. The simplest spin system belonged to an ethyl ester represented by a triplet methyl at δ0.95 coupled only to a quartet methylene at δ3.94, thus accounting for one degree of unsaturation. Although not recorded, it is likely, given standard laboratory methodology at the time, that the preservation solvent was ethanol. Presumably, this combined with the native carboxylate to form the ethyl ester, a phenomenon which seems to occur when algae are preserved in ethanol, for example, in the eicosanoids from *Lithothamnion* reported by Pietra.[124]

Two acetate singlets at δ1.70 and δ1.67 accounted for two additional degrees of unsaturation. A second spin system consisted of a triplet methylene at

C-2 (δ2.08) coupled to another methylene at δ1.58. The COSY spectrum clearly shows that this latter methylene is coupled to a diastereotopic methylene, one proton resonance of which is partially obscured by the C-3 methylene at 1.58. The other diastereotopic C-4 proton, however, is clearly visible at δ1.47, and is clearly coupled into the cluttered olefinic region. The need for an asymmetric center at C-5 (in the absence of one at C-3) places one of the acetates at C-5. Experience with 12-HEPE derivatives had shown that acetylation moves the α-hydroxyl proton into the olefinic region of the ^{1}H NMR spectrum. In the olefinic region, COSY analysis also showed a conjugated triene system which exhibited second-order coupled signals which were not easily interpreted. The other end of the carbon chain was composed of a triplet methyl at δ0.87 which was coupled into an overlapped methylene multiplet region. Following the spin system further was not possible, as it led into a complex, overlapped olefinic region.

A literature search uncovered a striking similarity between our spectrum and one published by Corey's group for 12-*epi*-(6*E*,8*Z*,10*E*,14*Z*)-leukotriene B_4 methyl ester diacetate.[218] Spectra for both compounds were run at high field strength in benzene-d_6. Except for the ester portion, all of our signals matched Corey's spectrum in lineshape and intensity, but not in chemical shift. All of the chemical shifts for protons in the carbon chains matched, except for the lowest field 2H multiplet, which Corey assigned to H-10 for the downfield portion and H-7 for the upfield portion. This multiplet was centered at δ6.90 in Corey's derivative, whereas an extremely similar 2H multiplet was centered at δ6.26 for compound **9**. We surmised that the triene moiety of compound **9** was not identical to Corey's leukotriene derivative, yet like Corey's contained an element of local symmetry.

In part, Corey's proton assignments were based on computer spectral simulation, which calculated an *EZE* conjugated triene system. Using the Bruker PANIC program for NMR spectral simulation, we were able to duplicate our NMR spectrum in this region for **9** using $J_{6,7} = J_{8,9} = J_{10,11} = 15$ Hz coupling constants, which would be expected in an all-*E* conjugated triene system. In addition to comparison with Corey's spectrum, placement of the conjugated triene

moiety at C-6 through C-11, thereby placing the other secondary acetate at C-12, is substantiated by the observed 15 and 7.5 Hz coupling constants for H-11. Moreover, the olefin chemical shifts and coupling constants for compound **9** are in agreement with those reported for methyl 5,12-dihydroxy-(6*E*,8*E*,10*E*,14*Z*)-eicosatetraenoate.[219]

Additional evidence for the all-*E* triene arrangement was deduced from the olefinic carbon chemical shifts as observed by the "distortionless enhancement by polarization transfer experiment" (DEPT). Seven olefinic carbons were clearly observed, indicating the presence of four olefins and accounting for all the remaining degrees of unsaturation in the molecule. The carbon at δ132.57 was significantly more intense than the other olefin signals; therefore, the resonances of two different carbons may have coincided at that frequency. Of the seven carbon lines observed, six were downfield of 130 ppm in $CDCl_3$, which in comparison to model conjugated triene systems,[206,220-221] was consistent with assignment to an all-*E* conjugated triene system. In these triene examples from the literature, the replacement of any *trans* olefin with *cis* geometry causes one or more olefinic carbons in the conjugated triene to appear upfield of 130 ppm in $CDCl_3$. By comparison to model compounds, the sole signal upfield of 130 ppm at δ123.32 is assigned to the non-conjugated olefin at C-14.[119-121,124,125,206,208] Compound **9** is therefore the diacetate, methyl ester derivative of (6*E*)-leukotriene B_4. Proton assignments for **9** followed from the combination of COSY data, spectral simulations, and the assignments for Corey's leukotriene deriviative.

Because derivative **9** was optically active, at least one of the stereocenters at C-5 and C-12 was not fully racemized. In the proton NMR spectrum, a singlet was observed 0.5 ppm downfield of each acetate signal at ca. 60% of the intensity of each of the major acetate peaks, perhaps signifying the co-existence of two diastereomers in solution. In view of the 12*S* stereochemistry of compounds **7** and **8** and the preponderence of 12*S*-lipoxygenase metabolism in the red algae,[52,208] it is likely that compound **9** is ethyl (5*RS*,12*S**)-diacetoxy-(6*E*,8*E*,10*E*,14*Z*)-eicosatetraenoate (6*E*-LTB_4). As depicted in Figure II.9, a mixture of epimers at C-5 would result from the non-enzymatic hydrolysis of an 11,12-LTA_4 intermediate

deriving from a 12*S*-hydroperoxide as was proposed by Westlund and coworkers for the metabolism of AA in human blood platelets.[223]

The isolation of 6*E*-LTB_4 from *M. periclados* is the first report of a leukotriene from a plant or marine source. There is one report of a potato lipoxygenase enzyme preparation converting exogenous AA to 6*E*-LTB_4 epimers at C-12;[224] however, these were not natural products. Moreover, within the plant kingdom, 20-carbon polyunsaturated fatty acid production is generally restricted to the algae.[20] Rat kidney homogenates produce 6*E*-LTB_4 as an enzymatic metabolite of LTB_4.[225] In vitro, 6*E*-LTB_4 induces chemokinesis in human polymorphonuclear leukocytes (PMNs) and the aggregation of rat PMNs. Further, it increases the vascular permeability of rabbit skin in vivo, but is not as potent as LTB_4.[226] The inflammatory response in animals may involve 6*E*-LTB_4; however, the function of it and all of the other eicosanoids in algae is unknown at this time.

Hydroxy-epoxy fatty acids.

In an effort to further explore the fatty acid metabolism of *Murrayella periclados*, we investigated more polar compounds than those isolated from the alga thus far. A collection from La Parguera, Puerto Rico in June 1988 was extracted and worked up in the usual manner. Polar VC fractions were combined, methylated, and subjected to a second VC. Fractions which eluted from this second column in 8-16% EtOAc contained a methylated mixture of UV-inactive compounds which were visualized with a unique TLC spray reagent. Originally developed by Andersen for the detection and identification of prostaglandins,[227] we have found cupric acetate in aqueous phosphoric acid to be an extremely sensitive indicator for fatty acids and their metabolites on TLC. The aforementioned fractions charred a brilliant blue color upon heating with this TLC spray reagent.

Further purification using a modification of Pace-Asciak's HPLC method for methylated hepoxilins[228] yielded four major compounds, all as optically active

oils. By ^{1}H NMR analysis, the least polar compound (**10**) was very similar to the acetate, methyl ester derivative of hepoxilin B_3, which had been isolated previously in our laboratory.[120] Direct comparison was not immediately possible because in the previous isolation, Moghaddam and Gerwick had worked exclusively with the acetate derivative. The α-hydroxy proton of compound **10** appeared as a broad multiplet at δ4.52, and the characteristic double doublet and double triplet of the epoxide, coupled by a 2 Hz *trans* coupling constant,[229] were transposed relative the acetate derivative.

The next compound (**11**) to elute from HPLC was similar to compound **10**, with an α-hydroxyl proton of the same shape as in **10** at δ4.52. The ^{1}H NMR spectrum of compound **11** differed from that of compound **10** in that the olefinic region was more congested and appeared by integration to contain an additional olefin. This was supported by the appearance of an additional allylic and an extra bis-allylic methylene in compound **11** and the abscence of a 6H multiplet at δ1.29 as compared to **10**. The IR spectra of these two compounds were practically identical and their optical rotations were both dextrorotatory and within 20% of each other in magnitude. Given the experimental errors involved with weighing of single milligrams and transferring solutions of a volatile solvent, the difference between the two optical rotations may be insignificant.

The third compound (**12**) to elute from the column appeared by ^{1}H NMR analysis to be similar to compound **10**, except that the α-hydroxyl proton appeared as a double double doublet at δ4.27. While the IR spectrum of **12** was essentially identical to **10** and **11**, the optical rotation was of the same magnitude but levorotatory. The most polar compound (**13**), was also strongly levorotatory, and its IR spectrum was indistinguishable from the other three compounds. Furthermore, the ^{1}H NMR spectrum of **13** displayed the α-hydroxyl proton at δ4.27 as did **12**, and also appeared to be an ω-3 polyunsaturated fatty acid metabolite. A COSY spectrum of derivative **13** (Figure II.6) revealed a terminal methyl triplet at δ0.89 coupled to an allylic methylene at δ1.96, which was in turn coupled to a signal in the crowded olefinic region, thus providing evidence for an ω-3 fatty acid. At the other end of the carbon chain, the COSY showed the

correlation pattern typical of a Δ-5 fatty acid.[120] Because of overlap in the olefinic region, the connectivity of the spin system in **13** could not be deduced between H-6 and H-18. It was apparent, however, that the allylic alcohol group was vicinal to a *trans*-epoxide (J = 2.1 Hz), which was coupled to a diastereotopic allylic methylene, a pattern seen in all four compounds in this series.

Because of the apparent similarity of this series of compounds to hepoxilin B_3, we made TMSi-ether derivatives of compounds **10-13** for GCMS analysis and comparison to the spectrum published by Walker et al. for the same derivative of hepoxilin B_3.[230] The mass spectra of the TMSi-ethers of both **10** and **12** displayed the characteristic peaks which identified them as diastereomeric TMSi-derivatives of hepoxilin B_3. In an earlier synthesis, Corey's group produced hepoxilin B_3 as a pair of diastereomers at C-10 which separated on TLC and HPLC.[231] Hepoxilin B_3 from *Murrayella* was subsequently acetylated as part of Moghaddam and Gerwick's structure elucidation of hepoxilin B_3 from *Platysiphonia miniata*.[120]

The mass spectrum of the TMSi-ether of compound **11** is reproduced in Figure II.7. It is extremely similar to the spectra for this derivative of hepoxilin B_3 except that the molecular ion is two mass units less, thus providing evidence for an ω-3 hepoxilin. The major fragmentions in the spectrum were at the same positions as reported for the methyl ester, *t*-butyldimethylsilyl ether derivative of hepoxilin B_4.[228] The characteristic cleavage at *m/z* 269 unequivocally places the allylic oxygen-bearing carbon at C-10. An identical mass spectrum was obtained for the TMSi-ether derivative of compound **13**, thus demonstrating that **11** and **13** were C-10 diastereomers of hepoxilin B_4. This ω-3 homolog is less well-known than hepoxilin B_3. Preliminary results show that hepoxilin B_4 is biosynthesized from EPA, and enhances the glucose-dependent release of insulin from pancreatic islet cells.[228]

This is the first report of hepoxilin B_4 from a plant or marine source, and the first recorded separation or NMR and polarimetric characterization of its two diastereomers. Even though the algal hepoxilins were isolated as methyl esters, they all contain a diastereotopic bis-allylic methylene at C-7, as do free acids **7** and **8**. However, when compound **7** was methylated to form derivative **5**, the C-7

proton signals collapsed into a non-diastereotopic pattern. These observations may reflect different conformations of these eicosanoid molecules in solution.

Although the separation of the hepoxilin B_3 diastereomers was accomplished in Corey's laboratory,[231] they were not sufficiently characterized for us to deduce the relative or absolute stereochemistry of our hepoxilins from *Murrayella*. For instance, optical rotations were not recorded. The assignment of relative stereochemistry of the *Murrayella* hepoxilins was based on a number of hydroxy-epoxy models from the literature. We may assume that the *erythro* and *threo* diastereomers exist in the conformations in the Newman projections proposed by Mercier and Agoh[232] and depicted in Figure II.8.

In a study of a series of hydroxy-epoxy compounds,[233] Mihelich made three important observations on the nature of diastereomers of this class. In all of the cases he examined, the *erythro* diastereomer had a higher R_f on TLC, i.e., was less polar. A physical basis for this observation may be explained by Hamberg's finding[155] that a diastereomer having two oxygens on the same side of the molecule (*threo*, in our case) was more polar than the diastereomer with oxygens on opposite sides (*erythro*). The dipoles of the opposing oxygens in the *erythro* isomer would tend to cancel each other out, resulting in a smaller dipole moment (less polarity) than the *threo* case.

Mihelich based his two remaining observations on ^{1}H NMR spectra. The α-hydroxyl proton of the *erythro* isomer, possibly due its the proximity to the electron-withdrawing epoxide oxygen, was consistently downfield from that of the *threo* isomer. Finally, the *erythro* α-hydroxyl proton couples to its vicinal epoxide proton by 3.2 Hz while the *threo* α-hydroxyl proton is coupled to its vicinal epoxide proton by $\geq$5 Hz. This phenomenon which was also reported independently by Mercier and Agoh.[234] Data for the algal hepoxilin B_3 and B_4 derivatives are consistent with the above arguments.

The same coupling constant analyses and literature examples we have used to establish the relative stereochemistries of our hydroxy-epoxy moieties were invoked by the Swedish and Dutch groups as well.[155,235] The ^{1}H NMR spectra of both diastereomers of hepoxilin B_4 are duplicated in Figure II.8 for the purpose of

comparison. The coupling constant and chemical shift trends expressed by Mihelich and others for *erythro* and *threo* diastereomerism in these systems has been verified by partial synthesis.[236]

Because we isolated 1:1 mixtures of hepoxilin B diastereomers from *M. periclados*, we likened their biosyntheses to that proposed by Pace-Asciak, who observed the same diastereomeric mixture in the production of hepoxilin B_3 by rat lung and bovine hemin preparations.[237] As depicted in the summary of *Murrayella* eicosanoid metabolism (Figure II.9), the stereochemistry created during initial hydroperoxide formation would be preserved during a hematin-assisted intramolecular hydroxyl transfer. In such a non-enzymatic hydroxyl tranfer, the hydroxyl could approach from either face of the diene, resulting in a 1:1 mixture of diastereomers. Recently, Hamberg proved that our *Murrayella*-derived compounds **10** and **13** both possess 12*S* stereochemistry. It follows that hepoxilin derivatives **11** and **12** are 12*S* as well.

As mentioned previously, a single diasteromer of the semi-synthetic acetate, methyl ester derivative of hepoxilin B_3 was isolated earlier in our laboratory from the red alga *Platysiphonia miniata*.[120] In that project, the assignment of the relative stereochemistry of the acetoxyl group to the epoxide was not attempted; however, based on the large coupling constants (9.2, 6.4 Hz) reported for the α-acetoxyl proton in that work, the relative stereochemistry of the *Platysiphonia* derivative should be assigned as *threo*. It is not known what effect, if any, acetylation has on the coupling constant in question, though it is probably minimal. Additional evidence for the assignment of *threo* stereochemistry for the *Platysiphonia* derivative is found in its carbon chemical shifts at C-10, 11, and 12, which are more in agreement with a *threo* rather than an *erythro* isomer.[236]

Curiously, both diastereomers of hepoxilin B_3 were present in *Murrayella*, while only one was isolated from *Platysiphonia*. However, it was reported that soybean lipoxygenase-1 enzymatically forms exclusively *threo*-11-hydroxy-*trans*-(12,13*S*)-epoxy-(9*E*)-octadecadienoic acid from (13*S*)-hydroperoxy-(9*Z*,11*E*)-octadecadienoic acid.[235] As reported in Chapter III of this thesis, an all *threo* series of hepoxilin-like compounds was isolated from the Oregon marine green

alga *Acrosiphonia coalita*, giving additional evidence for the preferential formation of *threo* hepoxilins in some marine algae.

Murrayella eicosanoid bioassay results.

The Syntex Research Corporation, Palo Alto, CA, performed enzyme inhibiton assays on some of the *Murrayella* eicosanoids. Experimental details are not included because some aspects of the assays may be proprietary. Results are presented in Table II.3. The initial assay measured inhibition of a canine renal Na^+/K^+ ATPase enzyme system, an ion pump which modulates kidney function. Different Na^+/K^+ ATPases are involved in heart and other organ functions, and are modulated by cardiac glycosides such as digitalis. The pharmaceutical industry continues to search for new, safe, and selective ATPase inhibitors to replace drugs like digitalis, for example, which may have serious side effects. A 1:1 mixture of trienes **2** and **3**, (as free acids from *Ptilota filicina*, Lopez and Gerwick, unpublished data) was only half as potent as 12*S*-HEPE (**7**) in inhibiting the renal Na^+/K^+ ATPase. In the bovine cornea, 12*R*-HETE, but not its epimer (**8**), inhibits Na^+/K^+ ATPase, and may help regulate of ocular transparency.[238]

Enzyme inhibition studies were also performed using an isolated H^+/K^+ ATPase from hog gastric mucosa. This ion pump helps maintain gastric fluid acidity. The only *Murrayella* eicosanoid tested, 12*S*-HEPE (**7**) was a weak inhibitor of this enzyme.

The human-derived 5-lipoxygenase enzyme initiates the classical route to the pro-inflammatory leukotrienes.[239] Drugs have have been formulated to inhibit this enzyme; however, none are currently in clinical use. Consequently, researchers continue to seek for more efficacious agents, and for a better understanding of the mechanisms and control features of the inflammatory diseases. Of all the algal eicosanoids tested, only 12*S*-HETE (**8**) significantly inhibited the human 5-lipoxygenase, possibly via a feedback mechanism. It has been shown that ω-3 15-lipoxygenase metabolites are slightly more potent inhibitors of the rat basophilic leukemia cell 5-lipoxygenase.[240]

Human phospholipase A_2 (PLA_2) is the final enzyme system for which inhibition assays were performed. This enzyme releases esterified arachidonate (AA) from phospholipids, after which AA can enter the lipoxygenase pathways. The speculation that 12*S*-HETE inhibits PLA_2 recently surfaced in the literature.[241] All the *Murrayella* eicosanoids that were tested inhibited PLA_2 at concentrations within the same order of magnitude. The 1:1 mixture of trienes **2** and **3** was more potent than the pure *EEE*-isomer **3**, possible evidence that the natural Δ-5 *E* olefin in these eicosanoids is responsible for the observed PLA_2 inhibition by the mixture. In this enzyme as well, feedback inhibition may occur.

Hepoxilin B methyl esters **10**, **11**, and **13** were provided to Dr. C.R. Pace-Asciak to determine if they stimulate the transport of calcium ions across the guinea pig yolk sac, as does hepoxilin A.[97] All three algal-derived compounds were inactive in this assay. This may be because hepoxilins of the B series are inactive, or because the free acid may be required for biological activity, perhaps via receptor binding. Many researchers have suggested that the unesterified carboxylate moiety is necessary for an eicosanoid to be biologically active, for example, in the binding of LTA_4 to LTA_4 epoxide hydrolase in dog lung.[242]

The antimicrobial principle(s) which were detected in crude extracts of *M. periclados* were not isolated or identified in this work. Neither compound **4**, **5**, nor **7** showed any significant antimicrobial activity in bioassays performed in our laboratory. However, we did not use the same test organism, the fungus *Cladosporium cucumerinum*, which Higgs used for detecting antimicrobial activity in the free acid from *Laurencia hybrida*[195] (**6**) which we subsequently revised to 12-HEPE (**7**).

EXPERIMENTAL

Ultraviolet (UV) absorption spectra were recorded on a Beckman DB-GT spectrophotometer. Infrared (IR) absorption spectra were recorded on either a Perkin-Elmer 727 or a Nicolet DXB FT 15 spectrophotometer. Optical rotations and optical rotatory dispersion (ORD) data were recorded on a Perkin-Elmer 141 polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM 400 spectrometer operating at a frequency of 400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C spectra, or on a Bruker ACP 300 spectrometer operating at a frequency of 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C acquisitions. All chemical shifts are reported relative to a tetramethylsilane (TMS) internal standard. All coupling constants are reported in Hertz (Hz), and are accurate to within 0.5 Hz. Low resolution mass spectra (LRMS) were obtained on a Finnigan 4023 spectrometer with both direct probe and gas chromatographic (GC) inlet capabilities. High resolution mass spectra (HRMS) were run on a Kratos MS 50 TC. MS ionizations were accomplished via electron impact (EI) or chemical ionization (CI, positive ion, CH_4). High performance liquid chromatography (HPLC) utilized standard Waters equipment, including M6000A and M45 pumps, U6K injector, R 401 differential refractometer and Lambda-Max Model 480 Variable Wavelength detectors. Thin layer chromatography (TLC) was performed using Merck aluminum-backed TLC sheets with fluorescent indicator (silica gel 60 F_{254}). Chromatography solvents were either HPLC-grade or distilled from glass prior to use. All liquid chromatographies utilized the normal phase mode.

Collection, extraction, and chromatography. Fine filaments of *Murrayella periclados* were scraped from intertidal mangrove aerial roots at Isla Guayacan off the southern coast of Puerto Rico in the summer months of 1984 and 1985. A voucher specimen has been deposited in the herbarium of the Department of Marine Sciences, University of Puerto Rico at Mayaguez. The algae (363 g dry wt.) was immediately packed in isopropanol (iPrOH), transported to Oregon, and stored frozen until workup. The alcohol was decanted, and the algae blended in a Waring blender in a 2:1 mixture of chloroform/methanol ($CHCl_3$/MeOH), after

which the solvent was removed by filtration. The resulting organic layer was collected in a separatory funnel, and combined with the organic layer resulting from gentle boiling of the algae in the same extraction solvent. The solvent was removed in vacuo, resulting in 8 g of crude organic extract. This extract was applied to a column of Merck silica gel G, and chromatographed in the vacuum mode[200-202] using a step gradient of increasingly polar mixtures of ethyl acetate (EtOAc) in 2,2,4-trimethylpentane (TMP).

Un-oxidized fatty acids. Vacuum chromatography (VC) fractions eluting in 30-40% EtOAc/TMP contained UV-active metabolites which on TLC analysis were observed to methylate with ethereal diazomethane (CH_2N_2). Therefore, these fractions were treated with CH_2N_2, resulting in 73 mg of dark oil which was subjected to a second VC. From this, the fraction eluting in 4% EtOAc/TMP contained the UV-active mixture (27 mg), which was injected onto HPLC (280 nm detection, 2 X Waters μPorasil 10 μm, 3.9 x 300 mm, 2% EtOAc/TMP). Three major compounds were purified, the least polar of which, methyl eicosapentaenoate, a colorless oil (5.3 mg, 0.06% yield), was not UV-active.

Methyl eicosapentaenoate (1). ^{1}H NMR (400 MHz, benzene-d_6): δ5.45 (9H, *m*, H-6, 8, 9, 11, 12, 14, 15, 17, 18), 5.29 (1H, *br dt*, J = 10.5, 7.3 Hz, H-5), 3.34 (1H, *s*, Me-ester), 2.85 (8H, *m*, H-7, 10, 13, 16), 2.10 (2H, *t*, J = 7.4 Hz, H-2), 2.00 (4H, *m*, H-4, 19), 1.60 (2H, *tt*, J = 7.4, 7.4 Hz), 0.91 (3H, *t*, J = 7.4 Hz). LR EIMS (probe) 70 eV, *m/z* (rel. int.): 318 $[M + 2 H]^+$ (5), 316 $[M]^+$ (3), 287 (3), 247 (6), 220 (6), 201 (12), 180 (13), 175 (13), 161 (14), 133 (29), 119 (48), 105 (54), 79 (100).

Methyl (5Z,7E,9E,14Z,17Z)-eicosapentaenoate (2). The next compound (4.2 mg, 0.05% yield) to elute from the HPLC column was UV-active and exhibited IR and ^{1}H NMR spectra identical with those previously reported for this compound.[206]

Methyl (5E,7E,9E,14Z,17Z)-eicosapentaenoate (3). The last compound recovered from this HPLC run (1.7 mg, 0.02% yield) was identified by matching UV and ^{1}H NMR spectra to those which had been previously published for this compound.[206]

Oxidized, UV-active fatty acid metabolites. From the original preparative-scale VC described previously, the fractions which eluted in 50-100% EtOAc in TMP contained a distinctive UV-active compound which tailed on TLC. In separate reactions, this substance reacted with both CH_2N_2 and with acetic anhydride in pyridine.

Methyl (12S)-acetoxyeicosapentaenoate (**4**). Material from crude VC fractions (125 mg) was methylated and acetylated as above, then purified by VC followed by preparative thick layer chromatography (pTLC, 2mm, 12% EtOAc in TMP) and HPLC (280 nm detection, Alltech Rsil 10 μm, 1 x 50 cm, 12% EtOAc in TMP) to yield 6 mg of a colorless oil (0.08% yield). IR ν_{max}^{CCl4} cm^{-1}: 3020, 2960, 2875, 1740, 1440, 1370, 1240. UV λ_{max}^{MeOH} nm: 238, (ε = 24,000). $[\alpha]_D$ -11° (acetone; *c* 0.47). For ^1H and ^{13}C NMR data, see Table III.1.

Methyl (12S)-hydroxyeicosapentaenoate (**5**). The original VC fraction which eluted in 100% EtOAc was treated with CH_2N_2 and subjected to a second VC. The fraction eluting from the column in 16% EtOAc in TMP contained a light yellow oil (9.3 mg, 0.12% yield). IR ν_{max}^{CCl4} cm^{-1}: 3450 (-OH), 3020, 2950, 2850, 1740, 1440, 1380, 1245, 985, 955. UV λ_{max}^{MeOH} nm: 236, (ε = 22,400). $[\alpha]_D$ +8° (acetone, *c* 0.87). For ^1H NMR data, see Table III.1.

Silylation of methyl ester **5**. One mg of **5** was dissolved in minimal silylation-grade pyridine and treated with an excess amount of N-trimethylsilylimidazole (TSIM) at room temperature for 20 min. to give the TMSi-ether derivative. This was then injected on GCMS equipped with a DB5 30W GLC column. The resulting EI spectrum was essentially identical to that published by Hamberg.[209] GC CIMS (methane) 70 eV *m/z* (rel. int.): 405 $[M + H]^+$ (33), 389 $[M - CH_3]^+$ (63), 315 $[M - OTMSi]^+$ (100), 295 $[M - CH_2CHCH(CH_2)_4CH_3]^+$ (83).

Chiral column analysis of **5**. The compound was purified on HPLC (237 nm detection, Nucleosil 100 5μm, 4.6 x 250 mm, 1% iPrOH in hexanes + 0.01% HOAc) and a single peak collected. This single peak was then injected onto chiral column HPLC (234 nm detection, Bakerbond DNBPG (ionic) 5 μm, 4.6 x 250 mm, 0.5% iPrOH in hexanes), from which a single peak eluted.

Purification and characterization of natural products. Original VC fractions enriched in polar, UV-active materials were applied to 2 mm pTLC plates and developed in hexane/diethyl ether/acetic acid (55:45:1). The UV-active band around 0.2 R_f, just below a prominent gray compound, was scraped from the plate, placed in a sintered glass funnel, and washed with excess diethyl ether (Et_2O) and EtOAc, which were removed in vacuo. The resulting oil was subjected to preparative HPLC (237 nm detection, Alltech Rsil 10 μm, 1 x 50 cm, hexane/ MeOH/ iPrOH/ HOAc 1050:26:22:1). Two major UV-active natural products were obtained, but baseline separation between the two was not achieved. Therefore, these fractions were purified separately using a slightly less polar solvent system for HPLC (Waters μPorasil 10 μm, 2 x [3.9 x 300 mm], hexane/ MeOH/ iPrOH/ HOAc 1070:20:16:1) to give free acids **7** and **8**. In collections of *M. periclados* from the wild, yields of compounds **7** and **8** ranged from 15 to 177 ppm and 13 to 87 ppm, respectively, on a dry weight basis.

(12S)-hydroxyeicosapentaenoic Acid (**7**). This free acid was the more polar of the two eluting from the HPLC system above. For ^1H NMR, see table III.1.

(12S)-hydroxyeicosatetraenoic Acid (**8**). ^1H NMR (400 MHz, benzene-d_6): δ6.83 (1H, *dd*, J = 15, 11 Hz, H-10), 6.02 (1H, *dd*, J = 11, 11 Hz, H-9), 5.61 (1H, *dd*, J = 15, 6 Hz, H-11), 5.52 (1H, *m*), 5.42 (3H, *m*), 5.25 (1H, *m*, H-5), 4.21 (1H, *bdt*, J = 6, 6 Hz, H-12), 3.04 (1H, *ddd*, J = 15, 7.5, 7.5 Hz, H-7a), 2.82 (1H, *ddd*, J = 15, 7.5, 7.5 Hz, H-7b), 2.31 (1H, *ddd*, J = 14, 7, 7 Hz, H-13a), 2.24 (1H, *ddd*, J = 14, 7, 7 Hz, H-13b), 2.10 (6H, *m*, H-2, 4, 16), 1.50 (2H, *tt*, J = 7.2, 7.2 Hz, H-3), 1.25 (6H, *m*, H-17, 18, 19) 0.89 (3H, *t*, J = 7.2 Hz, H-20). ORD: $[\alpha]_D^{27}$ +19°, $[\alpha]_{546}^{27}$ +25°, $[\alpha]_{436}^{27}$ +45° (*c* 0.16, acetone). The NMR spectrum also appears in Figure II.3.

Methylation and silylation of **8**. One mg of **8** was treated with CH_2N_2 to form the methyl ester, then silylated as above for methyl ester **5**. The EI GCMS spectrum was effectively identical to that published for the same derivative by Samuelsson and Hamberg.[216]

Collection, extraction, and chromatography for (6E)-leukotriene B_4 ethyl ester diacetate. *M. periclados* (56 g dry wt) was collected in the fall of 1984 from

mangroves near La Parguera, Puerto Rico, and preserved in an unspecified alcohol (MeOH or EtOH). Alcohol was decanted and algae extracted 3X with (0.5 l per extraction) 2:1 $CHCl_3$/MeOH to yield 2.46 g of crude lipid extract, which was vacuum chromatographed. The fraction eluting in 40% EtOAc was inactive in an assay for inhibition of hog gastric mucosa H^+/K^+ ATPase, but contained a polar UV-active metabolite by TLC analysis. This TLC spot was not observed to methylate, but did acetylate. Therefore, the entire fraction was acetylated overnight in 1:1 acetic anhydride/pyridine. The solvent was removed in vacuo, and the acetylated fraction was subjected to a second VC. From this, the fraction eluting in 10% EtOAc in TMP (27 mg) contained the compound of interest. This entire fraction was injected on HPLC (254 nm detection, Versapack 10μm, 2 x [300 x 4.1 mm], 12% EtOAc in n-heptane). The major UV-active compound was repurified twice more on HPLC (270 nm detection, 0.5% iPrOH in hexane) to yield 2 mg (0.08% yield) of compound **9** as a clear orange-colored oil.

(6E)-leukotriene B_4 ethyl ester diacetate (**9**). FTIR ν_{max}^{film} cm^{-1}: 2956, 2928, 2856, 1737, 1237, 1183, 1175, 1019, 1000. UV λ_{max}^{MeOH} nm: 260, 270, 281 (ε = 30,000; 40,000; 32,000). $[\alpha]_D$ -3.6° (*c* 0.25, acetone). ^{1}H NMR (400 MHz, benzene-d_6): δ6.29 (1H, *m*, J = 15, 11 Hz, H-10), 6.23 (1H, *m*, J = 14.9, 11 Hz, H-7), 6.02 (1H, 2nd order *m*, J = 15, 11 Hz, H-9), 5.99 (1H, 2nd order *m*, H-8), 5.60 (1H, *dd*, J = 15, 7.5 Hz, H-11), 5.46 (3H, *m*, H-6, 12, 15), 5.40 (2H, *m*, H-5, 14), 3.94 (2H, *q*, J = 7.2 Hz, ethyl ester), 2.48 (1H, *ddd*, J = 14, 7, 7 Hz, H-13a), 2.33 (1H, *ddd*, J = 14, 7, 7 Hz), 2.08 (2H, *t*, J = 6.9 Hz, H-2), 2.00 (2H, *dt*, J = 7, 7 Hz, H-16), 1.70 (3H, *s*, -OAc), 1.67 (3H, *s*, -OAc), 1.58 (3H, *m*, H-3, 4a), 1.47 (1H, *m*, H-4b), 1.27 (6H, *m*, H-17, 18, 19), 0.95 (3H, *t*, J = 7.2 Hz, ethyl ester), 0.87 (3H, *t*, J = 6.8 Hz, H-20). ^{13}C NMR (100.61 MHz, $CDCl_3$) 133.29 (CH), 132.84 (CH), 132.57 (2 x CH), 132.27 (CH), 131.72 (CH), 131.50 (CH), 123.32 (CH), 73.90 (2 x CH), 60.37 (CH_2), 33.88 (CH_2), 33.75 (CH_2), 32.40 (CH_2), 31.50 (CH_2), 29.20 (CH_2), 27.38 (CH_2), 22.58 (CH_2), 21.28 (2 x CH_3), 20.57 (CH_2), 14.24 (CH_3), 14.07 (CH_3). Carbon multiplicities were determined by a DEPT experiment. EIMS 70 eV *m/z* (rel. int.): 448 $[M]^+$ (0.07), 406 (0.4), 388 $[M - HOAc]^+$ (15), 346 $[M - HOAc - H_2C{=}C{=}O]^+$ (61), 329 $[M - HOAc - OAc]^+$ (10), 235 (85), 217

(23), 189 (64), 143 (85), 129 (69), 43 $[COCH_3]^+$ (100). HR EIMS *m/z* obs. $[M]^+$ 448.2825 ($C_{26}H_{40}O_6$, 0.02 mmu dev.)

Collection, extraction, and chromatography of hepoxilins. M. periclados (184 g dry wt.) was re-collected in June 1988 from mangroves near La Parguera, Puerto Rico. The iPrOH-preserved algae was extracted with 2:1 dichloromethane (CH_2Cl_2)/ MeOH in the standard manner, resulting in 6 g of crude organic extract. VC was performed using a step-gradient of EtOAc in n-heptane. Fractions eluting from 40 to 100% EtOAc (178 mg) were combined, methylated with CH_2N_2, and vacuum chromatographed again using a step gradient of EtOAc in cyclohexane. Fractions eluting from 8-16% contained a UV-inactive, methylated mixture of compounds (20 mg) which charred a brilliant blue color on TLC after spraying the developed plate with cupric acetate in aqueous phosphoric acid solution.[227] This material was separated on HPLC (refractive index detection, Nucleosil 100 5 μm, 4.6 x 250 mm, 1% iPrOH in hexanes + 0.01% HOAc). Four major compounds (**10** - **13**) eluted from the column with almost baseline separation. A 100-200 μg portion of each compound was separately dissolved in silylation-grade pyridine and treated with TSIM at room temperature for 20 minutes to make the four TMSi-ether derivatives for LR GC CIMS analysis.

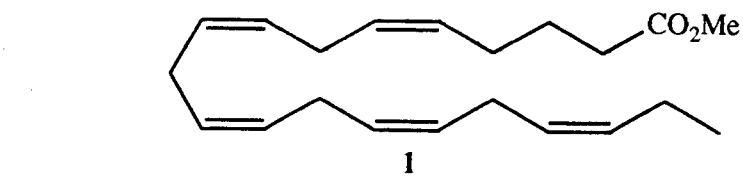
Erythro hepoxilin B_3 methyl ester (**10**). FTIR ν_{max}^{film} cm^{-1}: 3430 (br -OH), 3013, 2955, 2928, 2856, 1739, 1438, 1200, 1157. $[\alpha]_D$ +40° (*c* 0.11, acetone). ^1H NMR (300 MHz, benzene-d_6): δ5.45 (4H, *m*, H-8, 9, 14, 15), 5.34 (1H, *br dt*, J = 10, 7 Hz, H-6), 5.28 (1H, *br dt*, J = 10, 7 Hz, H-6), 4.52 (1H, *m*, H-10), 3.34 (3H, *s*, Me-ester), 2.99 (1H, *dt*, J = 5, 2 Hz, H-12), 2.76 (1H, *m*, H-11), 2.73 (2H, *m*, H-7), 2.24 (2H, *m*, H-13), 2.07 (2H, *t*, J = 7.2 Hz, H-2), 1.94 (4H, *m*, H-4, 16), 1.77 (1H, *br d*, J = 2.5 Hz, OH), 1.55 (2H, *tt*, J = 7.2, 7.2 Hz, H-3), 1.29 (6H, *m*, H-17, 18, 19), 0.87 (3H, *t*, J = 6.7 Hz). TMSi-ether GC CIMS 70 eV *m/z* (rel. int.): 423 $[M + H]^+$ (0.03), 407 $[M + H - CH_3]^+$ (27), 333 $[M - OTMSi]^+$ (27), 315 (21), 283 (100), 269 (47), 161 (28). Although a different ionization mode was used by Walker et al. for the same derivative,[230] spectra for both isolates were comparable.

Erythro hepoxilin B_4 methyl ester (**11**). FTIR ν_{max}^{film} cm^{-1}: 3430 (br -OH), 3013, 2961, 2933, 1738, 1438, 1200, 1157, 721. $[\alpha]_D$ +31° (*c* 0.27, acetone). ^1H

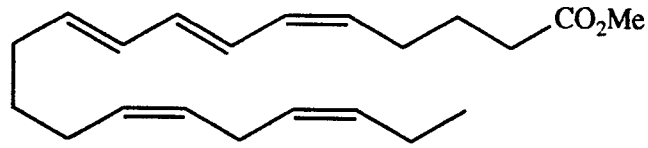
NMR (300 MHz, benzene-d_6): δ5.49 (1H, *br dt*, J = 9.6, 6.8 Hz, H-9), 5.42 (5H, *m*, H-8, 14, 15, 17, 18), 5.33 (1H, *m*, H-6), 5.28 (1H, *br dt*, J = 9, 7 Hz, H-5), 4.51 (1H, *br ddd*, J = 6.8, 3.4, 2.7 Hz, H-10), 3.34 (3H, *s*, Me-ester), 2.98 (1H, *dt*, J = 6, 2.2 Hz, H-12), 2.79 (1H, *m*, H-7a), 2.75 (1H, *dd*, J = 3.4, 2.2 Hz, H-11), 2.70 (2H, *br dd*, J = 6, 6 Hz), 2.65 (1H, *m*, H-7b), 2.24 (1H, *ddd*, J = 14, 7, 6 Hz, H-13a), 2.18 (1H, *ddd*, J = 14, 7, 6 Hz, H-13b), 2.07 (2H, *t*, J = 7.3 Hz, H-2), 1.96 (2H, *dt*, J = 7.4, 7.4 Hz, H-19), 1.90 (2H, *m*, H-4), 1.79 (1H, *d*, J = 2.7 Hz, -OH), 1.55 (2H, *tt*, J = 7.4, 7.4 Hz, H-3), 0.89 (3H, *t*, J = 7.4 Hz, H-20). For LR GC CIMS of the TMSi-ether derivative, see Figure III.7.

Threo hepoxilin B_3 methyl ester (**12**). FTIR and GC CIMS: Identical to diastereomer **10**. $[\alpha]_D$ -39° (*c* 0.15, acetone). ^{1}H NMR (300 MHz, benzene d_6): δ5.40 (6H, *m*, H-5, 6, 8, 9, 14, 15), 4.27 (1H, *ddd*, J = 8, 5.1, 5.2 Hz, H-10), 3.33 (3H, *s*, Me-ester), 2.91 (1H, *dt*, J = 5.4, 2.1 Hz, H-12), 2.78 (1H, *dd*, J = 5.1, 2.1 Hz, H-11), 2.68 (2H, *m*, H-7), 2.22 (2H, *m*, H-13), 2.06 (2H, *t*, J = 7.3 Hz, H-2), 1.92 (4H, *m*, H-4, 16) 1.83 (1H, *d*, J = 5.2 Hz, -OH), 1.54 (2H, *tt*, J = 7.3, 7.3 Hz, H-3), 1.22 (6H, *m*, H-17, 18, 19), 0.87 (3H, *t*, J = 6.8 Hz, H-20).

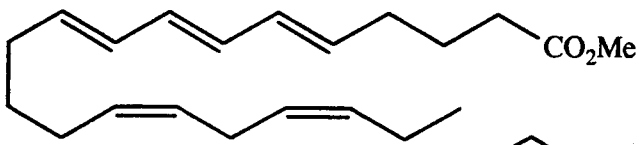
Threo hepoxilin B_4 methyl ester (**13**). FTIR and GCMS: Identical to diastereomer **11**. $[\alpha]_D$ -47° (*c* 0.27, acetone). ^{1}H NMR (300 MHz, benzene d_6): δ5.38 (8H, *m*, H-5, 6, 8, 9, 14, 15, 17, 18), 4.27 (1H, *ddd*, J = 8, 5.2, 4.9 Hz, H-10), 3.33 (3H, *s*, Me-ester), 2.90 (1H, *dt*, J = 6, 2.1, H-12), 2.81 (1H, *ddd*, J = 16, 8, 8 Hz, H-7a), 2.76 (1H, *dd*, J = 4.9, 2.1 Hz, H-11), 2.71 (1H, *bt*, J = 6.3 Hz, H-16), 2.66 (1H, *ddd*, J = 16, 8, 8 Hz), 2.26 (1H, *ddd*, J = 14, 7, 6 Hz, H-13a), 2.15 (1H, *ddd*, J = 14, 7, 6 Hz, H-13b), 2.06 (2H, *t*, J = 7.3 Hz, H-2), 1.96 (2H, *m*, H-19), 1.90 (2H, *m*, H-4), 1.86 (1H, *d*, J = 5.2 Hz, -OH), 1.54 (2H, *tt*, J = 7.3, 7.3 Hz, H-3), 0.89 (3H, *t*, J = 7.6 Hz, H-20).



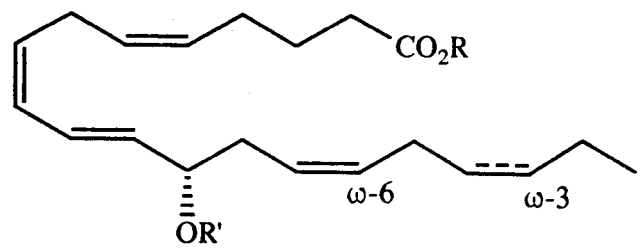
1



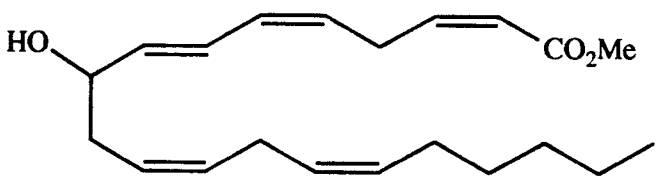
2



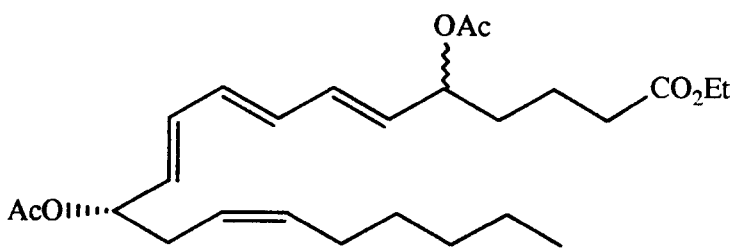
3



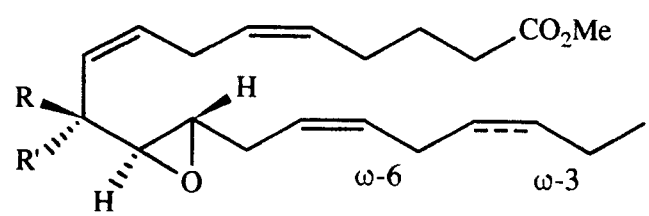
4 R = Me, R' = Ac, ω-3
5 R = Me, R' = H, ω-3
7 R = H, R' = H, ω-3
8 R = H, R' = H, ω-6



6



9



10 R = OH, R' = H, ω-6
11 R = OH, R' = H, ω-3
12 R = H, R' = OH, ω-6
13 R = H, R' = OH, ω-3

Figure II.1. Chemical structures for Chapter II.

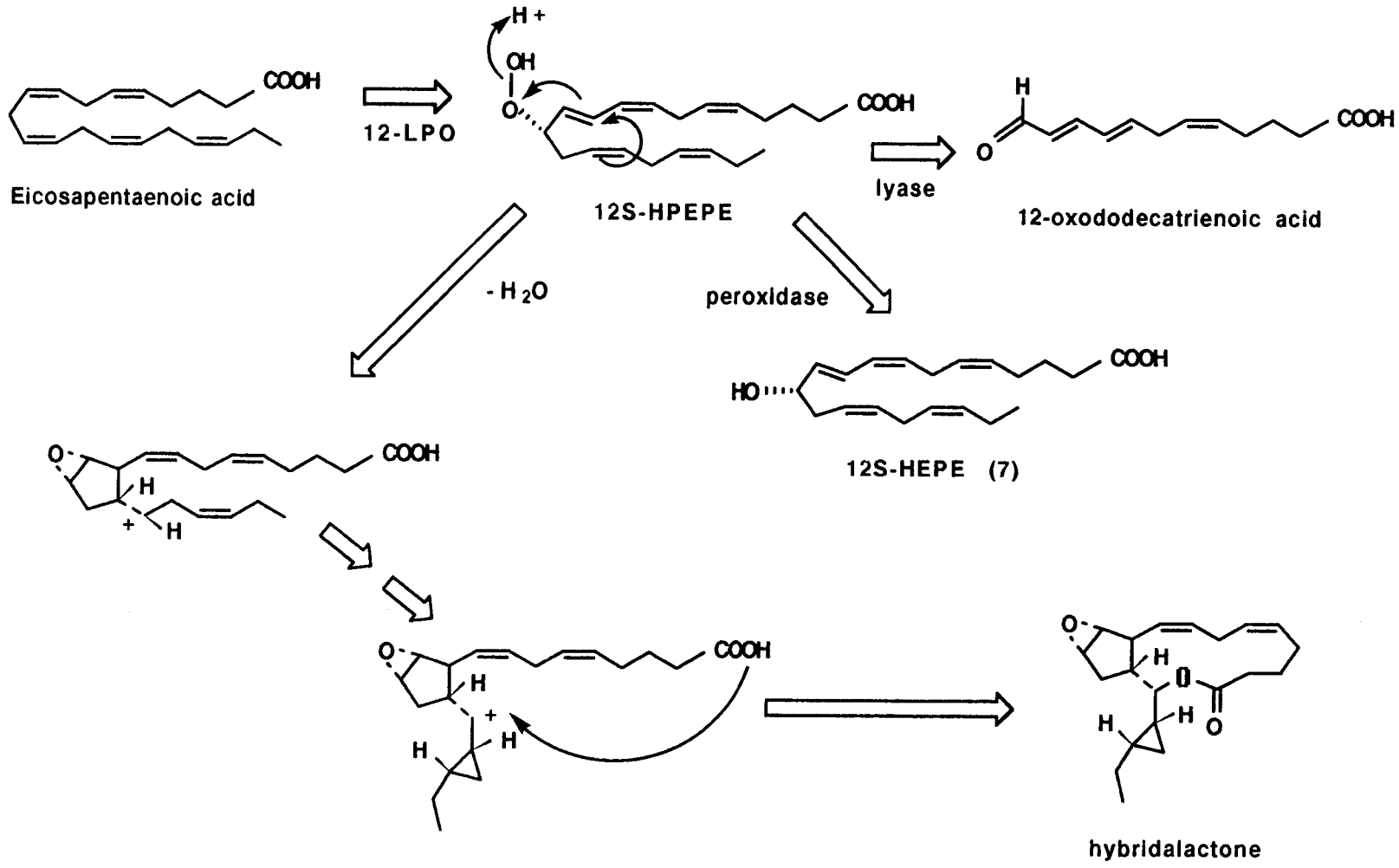


Figure II.2. 12-Lipoxygenase metabolism in *Laurencia hybrida*, adapted in part from reference 198.

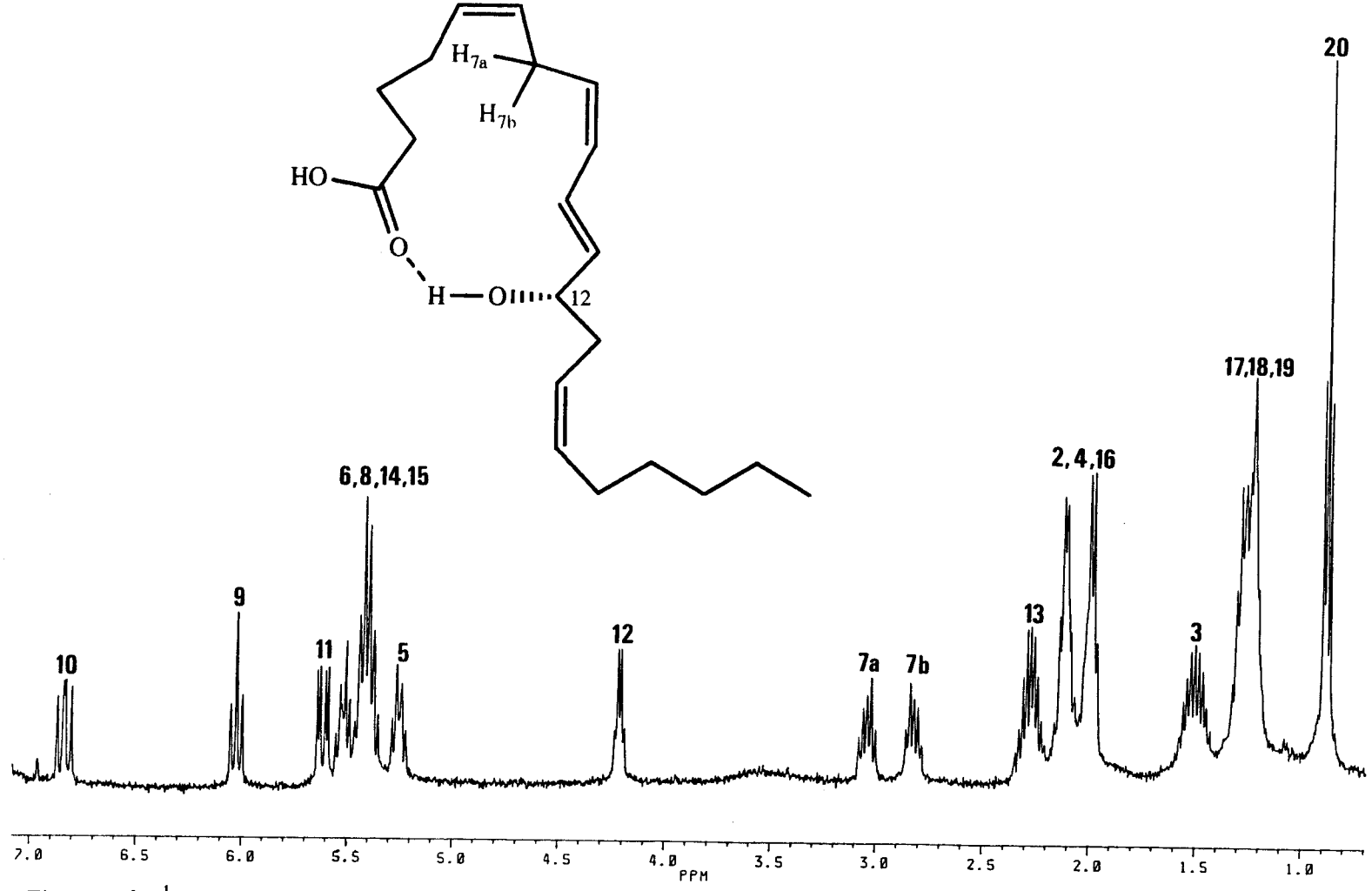


Figure II.3. ^{1}H NMR spectrum and proposed cyclized solution conformation of 12S-HETE (**8**).

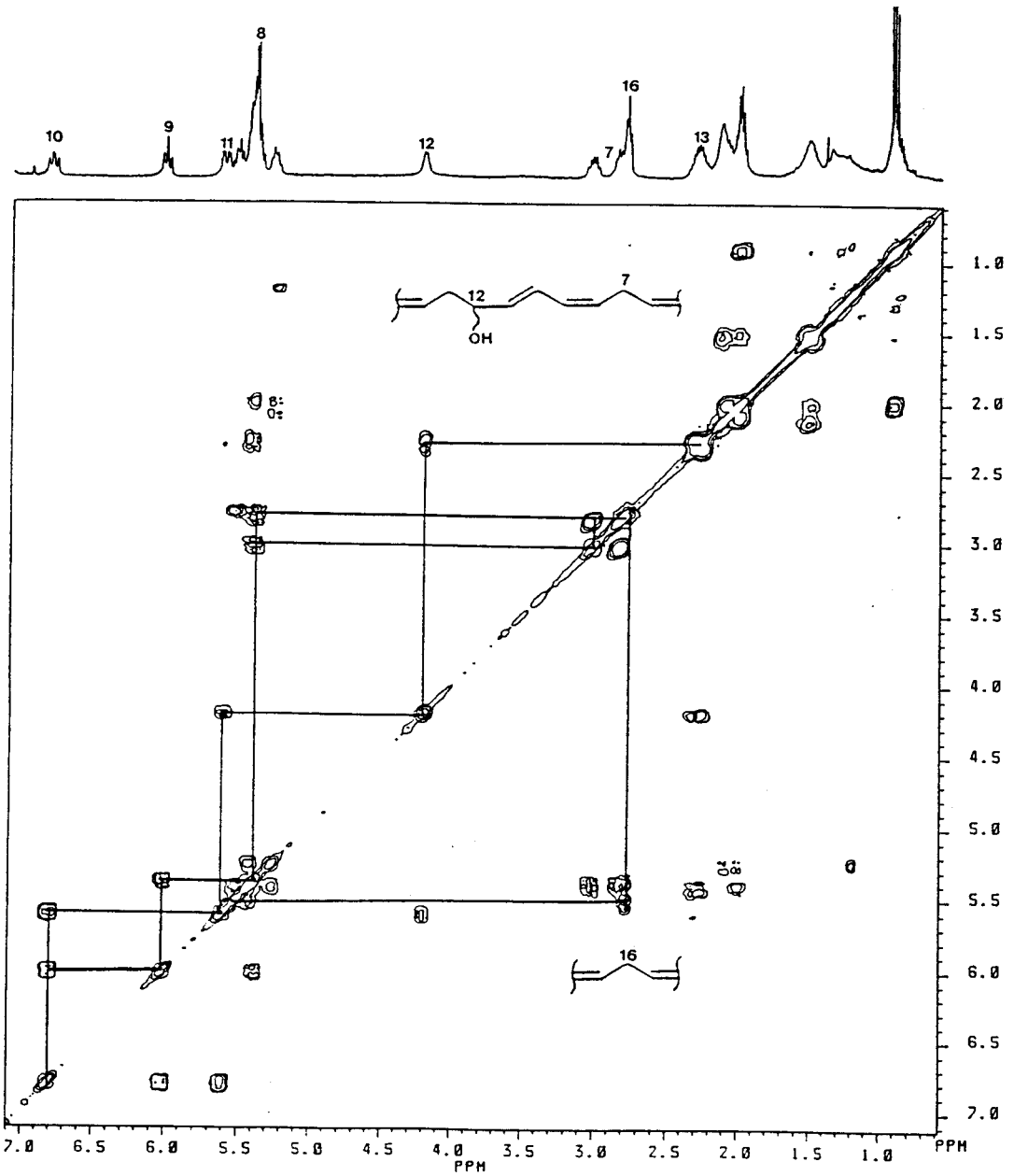


Figure II.4. ^{1}H-^{1}H COSY of 12*S*-HEPE showing correlations between coupled protons.

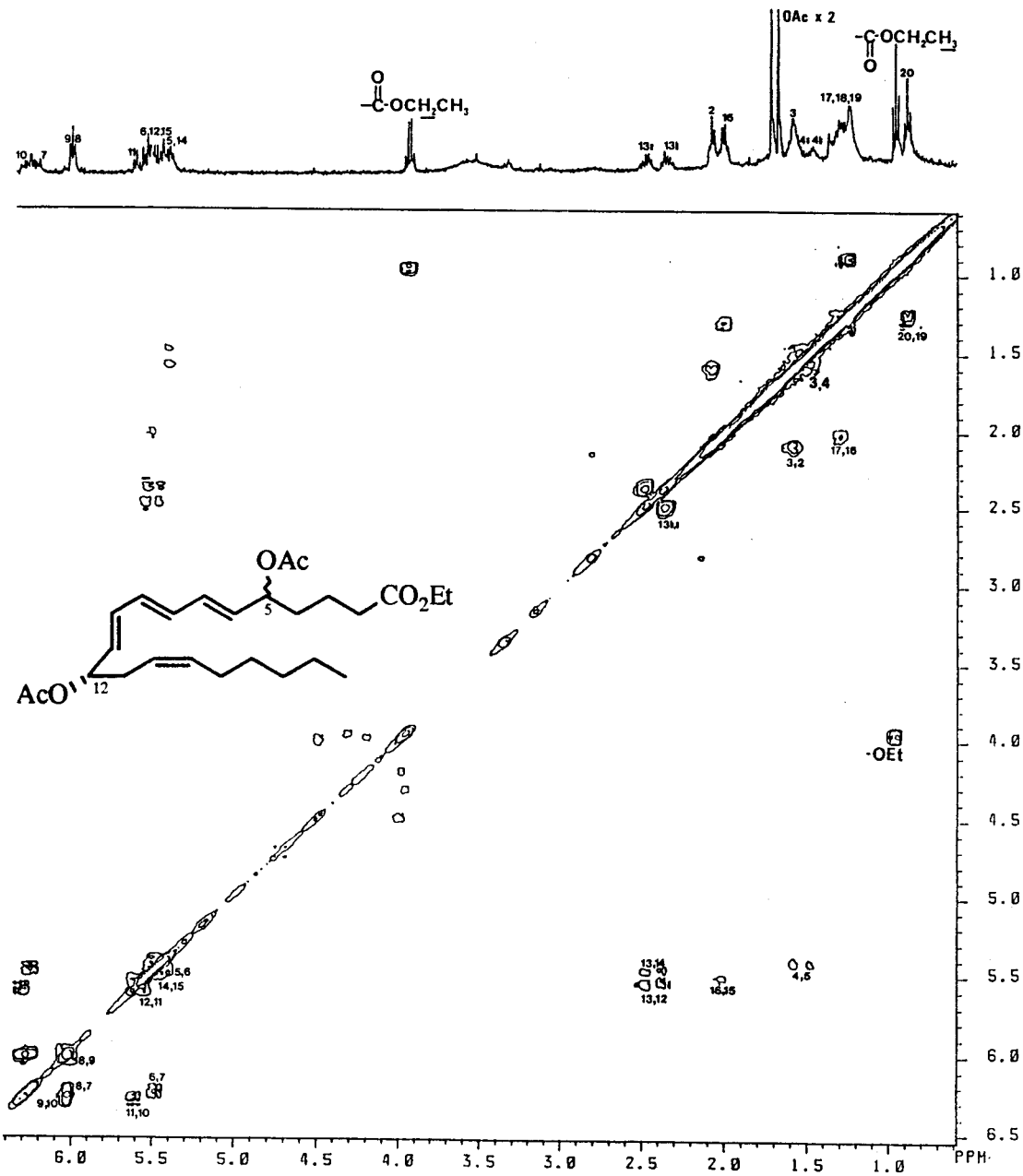


Figure II.5. ^{1}H-^{1}H COSY of 6*E*-LTB_4 derivative (**9**) with labelled correlations between coupled protons.

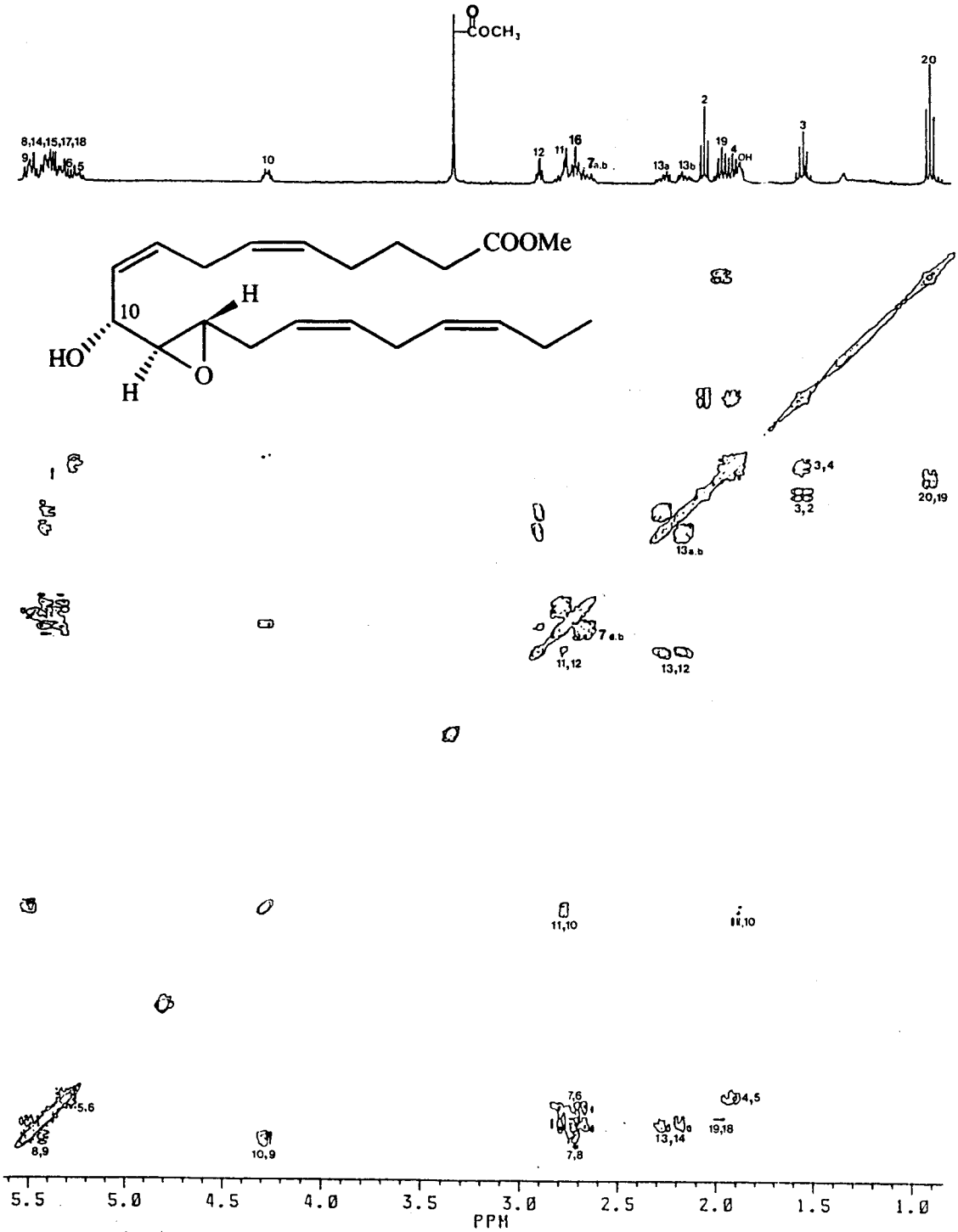


Figure II.6. ^{1}H-^{1}H COSY of *threo* hepoxilin B_4 methyl ester (**13**) with labelled correlations between coupled protons.

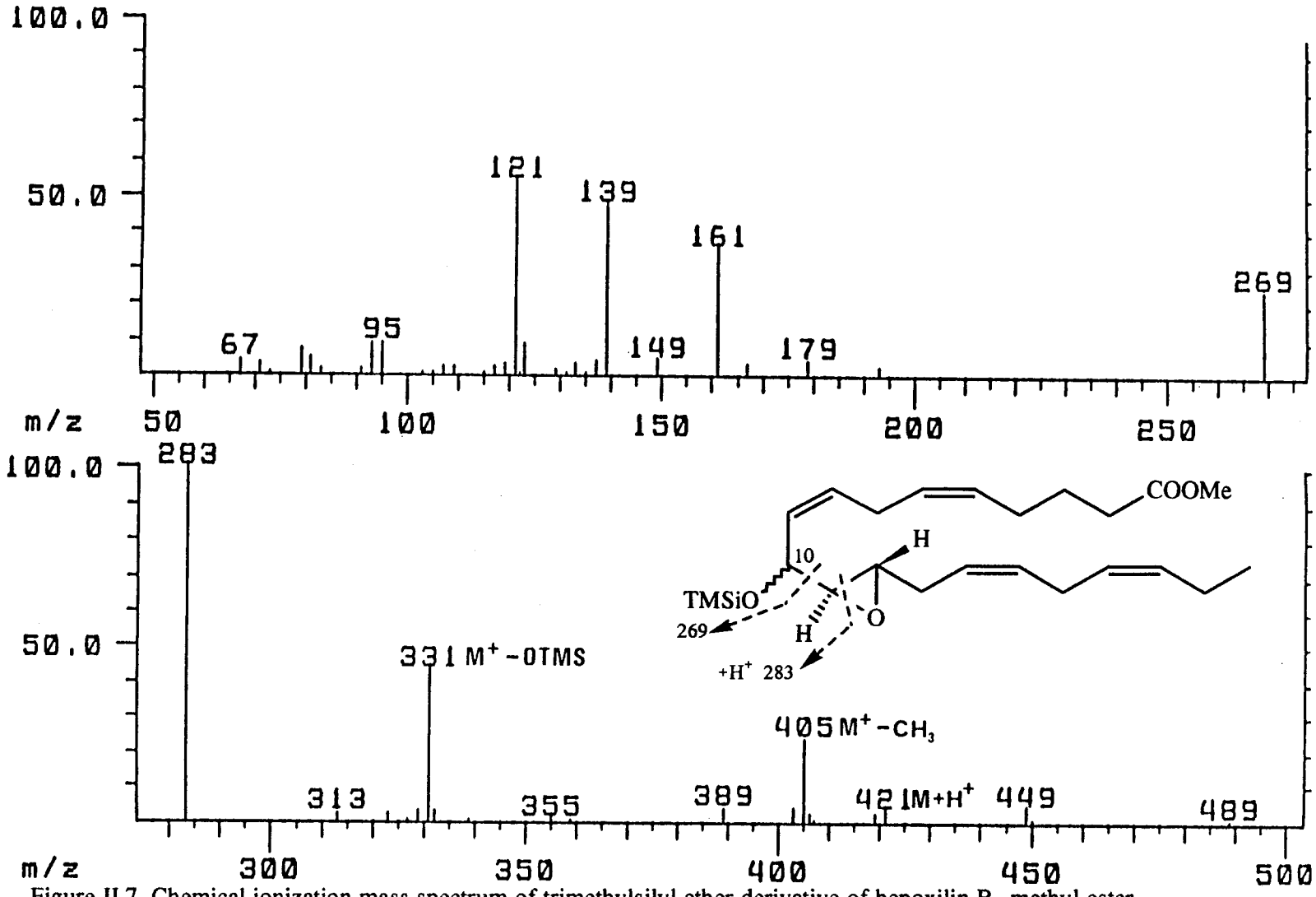


Figure II.7. Chemical ionization mass spectrum of trimethylsilyl ether derivative of hepoxilin B_4 methyl ester.

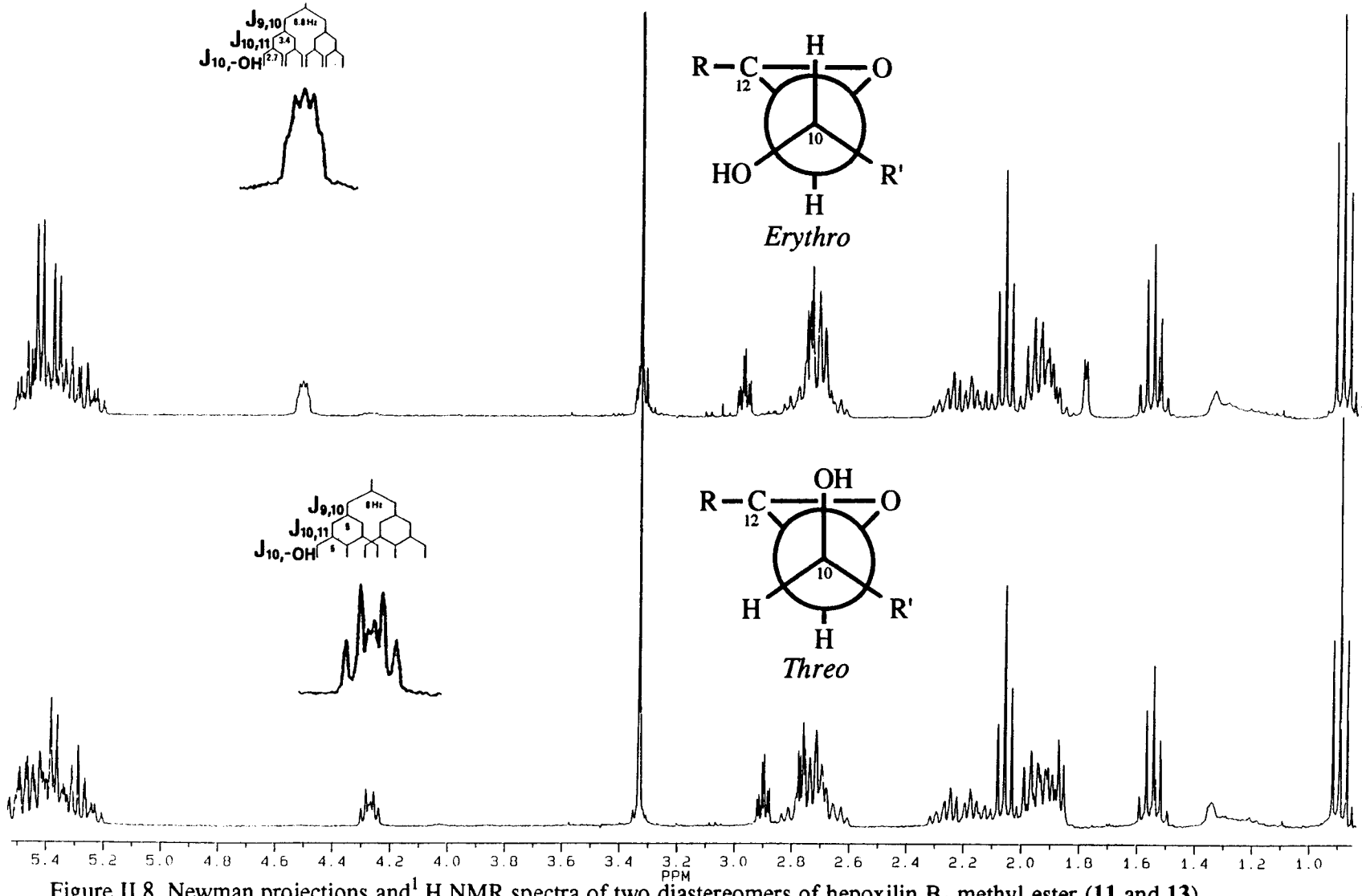


Figure II.8. Newman projections and[1] H NMR spectra of two diastereomers of hepoxilin B_4 methyl ester (**11** and **13**).

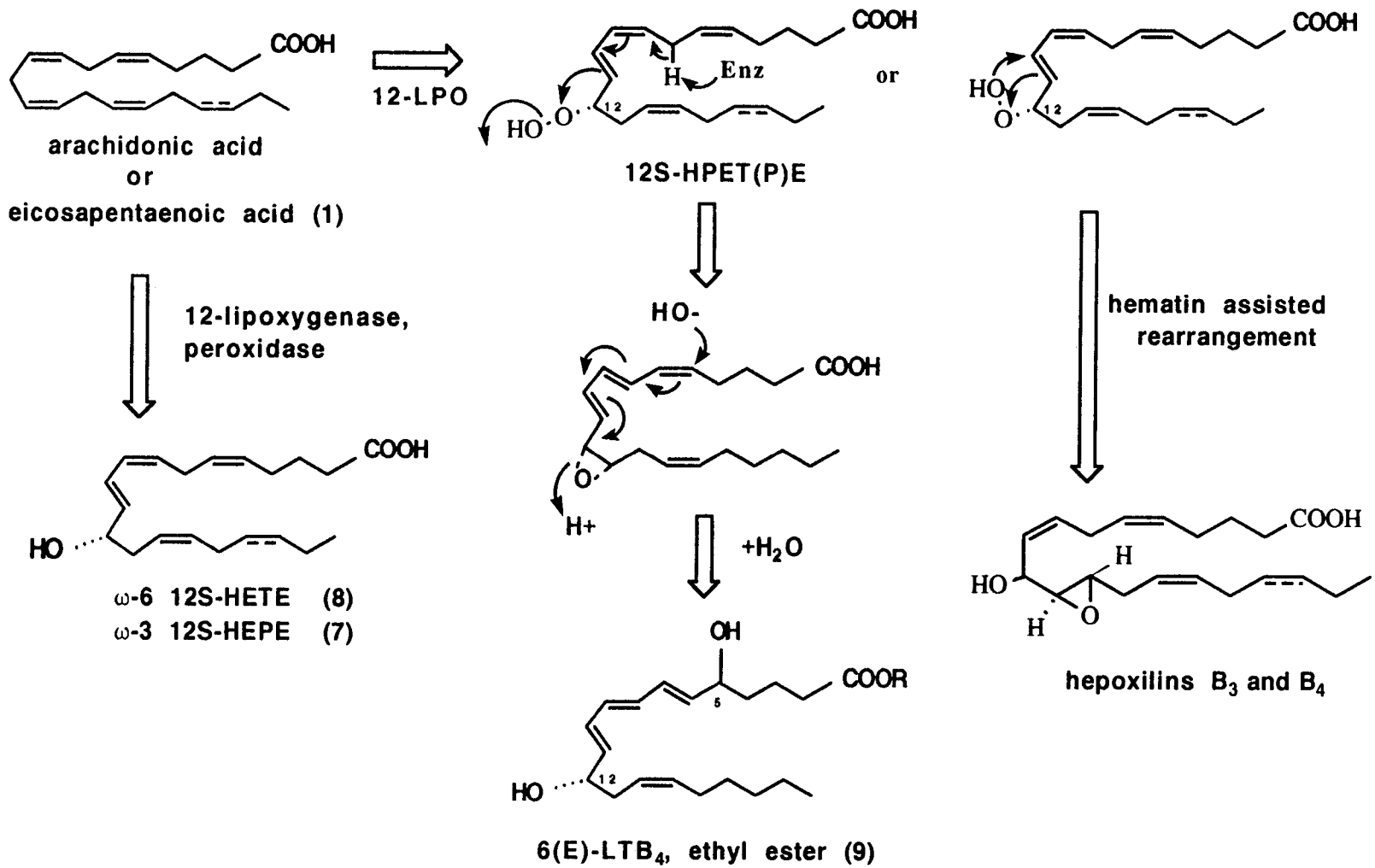


Figure II.9. Eicosanoid metabolism in *Murrayella periclados*.

Table II.1 NMR Data for 12*S*-hydroxyeicosapentaenoic Acid and Two Derivatives from *Murrayella periclados*[a]

	Methyl Ester Acetate 4[b]				Methyl Ester 5			Natural Product 7[c]		
C#	δ ^{13}C	δ ^{1}H	m	J (Hz)	δ ^{1}H	m	J (Hz)	δ ^{1}H	m	J (Hz)
1	173.14	----	-	---	----	-	---	----	-	---
2	33.33	2.10	t	7.4	2.07	t	7.3	2.00	t	6.7
3	25.02	1.58	tt	7.4, 7.4	1.56	tt	7.3, 7.3	1.52	m	---
4	26.75	1.93	dt	7.4, 7.4	1.94	dt	7.3, 7.3	2.00	m	---
5	129.76	5.26	bdt	10, 7.4	5.27	bdt	10, 7.3	5.26	m	---
6	127.21[d]	5.39	m	---	5.39	m	---	5.40	m	---
7	26.41	2.83	dd	7.4, 7.4	2.89	dd	5.8, 5.8	3.03	ddd	16, 8, 8
								2.83	ddd	16, 8, 8
8	131.49[d]	5.39	m	---	5.39	m	---	5.40	m	---
9	128.2	5.97	dd	11.1, 11.1	6.04	dd	11, 11	5.99	dd	11, 11
10	128.3	6.74	dd	14.0, 11.1	6.72	dd	11, 15	6.81	dd	15, 11
11	131.75	5.60	m	---	5.67	dd	15, 6.5	5.61	dd	15, 5.6
12	74.03	5.51	m	---	4.13	bdt	7, 6.5	4.21	bdt	7, 5.6
13	32.92	2.49	ddd	14, 7, 7	2.36	ddd	14, 7, 7	2.30	ddd	14, 7, 7
		2.36	ddd	14, 7, 7	2.30	ddd	14, 7, 7	2.26	ddd	14, 7, 7
14	131.37	5.51	m	---	5.51	m	---	5.41	m	---
15	128.4[d]	5.39	m	---	5.39	m	---	5.41	m	---
16	26.05	2.79	dd	6.6, 6.6	2.80	dd	6.0, 6.0	2.77	dd	6.5, 6.5
17	124.47	5.45	m	---	5.51	m	---	5.40	m	---
18	132.26[d]	5.39	m	---	5.39	m	---	5.40	m	---
19	20.86	2.00	dq	7.2, 7.2	1.99	dq	7.4, 7.0	2.00	m	---
20	14.36	0.92	t	7.2	0.90	t	7.4	0.91	t	7.6
1'	50.90	3.37	s	---	3.34		---	----	-	---
12'	20.78	1.72	s	---	---		---	----	-	---
	169.40									

[a]All spectra were obtained at 9.398 T in C_6D_6. Chemical shifts are expressed in ppm relative to TMS internal standard.
[b]Assignments based on COSY and HETCOR experiments and comparison to a model compound.[118]
[c]Assignments based on a COSY experiment.
[d]Assignments may be interchanged.

Table II.2 Comparison of ^1H NMR Spectrum of 12*S*-HEPE Methyl Ester with Data for an Unusual Eicosanoid from *Laurencia hybrida*[a]

Δ-5, ω-3, 12-S-HEPE Methyl Ester (5)[b]					Purported Δ-2 9-HEPE Methyl Ester (6)[c]				
C#	δ ^1H	# H's	m	J (Hz)	C#	δ ^1H	# H's	m	J (Hz)
10	6.55	1H	bdd	11, 15	7	6.56	1H	ddt	11, 15, 1
9	5.99	1H	bdd	11, 11	6	5.99	1H	tt	11, 1
11	5.73	1H	dd	6, 15	8	5.73	1H	dd	7, 15
15	5.55[d]	1H	bdt	11, 6	12	5.56	1H	dtt	11, 7, 1
6,8,14 17,18	5.40	5H	m	---	11	5.44	1H	dtt	11, 7, 1
					2,3,5,15	5.38	4H	m	---
5	5.30	1H	bdt	10, 7	14	5.30	1H	dtt	11, 7, 1
12	4.25	1H	dt	6, 6	9	4.24	1H	q	7, 7
1'	3.67	3H	s	---	1'	3.67	3H	s	---
7	2.93	2H	bdd	6, 6	4	2.91	2H	bt	7, 7, 1
16	2.81	2H	bdd	6, 6	13	2.80	2H	bt	7, 7, 1
13	2.37	1H	ddd	12, 6, 6	---	----	--	-	---
2	2.33	2H	t	7	10	2.38	2H	ABMX	---
13	2.32	1H	ddd	12, 6, 6	18	2.32	2H	p	7, 7
4	2.11	2H	dt	7, 7	16	2.10	2H	p	7, 7
19	2.06	2H	dq	7, 7	19	2.06	2H	sextet	7, 7
3	1.70	2H	tt	7, 7	17	1.76	2H	p	7, 7
20	0.97	3H	t	7	20	0.96	3H	t	7

[a]Higgs, M.D., reference 195.
[b]Spectrum acquired in $CDCl_3$ at 400 MHz with TMS as an internal standard.
[c]Spectrum acquired in $CDCl_3$ at 360 MHz with TMS as an internal standard.
[d]Assignment may be interchanged with a proton in the multiplet at δ5.40.

Table II.3 Algal Eicosanoid Enzyme Inhibition Assays (Syntex Research)

	IC_{50} (μM)[1]				
Enzyme System	Trienes (**2** and **3**) 1:1 mix	*EEE* triene Methyl ester (**3**)	12*S*-HEPE (**7**)	12*S*-HETE (**8**)	*Threo* Me-Hepoxilin B_3 (**12**)
Na^+/K^+ ATPase[2]	60 (free acids)	NA[6]	30	NA	NA
H^+/K^+ ATPase[3]	NA[6]	NA	30	NA	NA
5-LO-ase[4]	NA	inactive	inactive	>60 (weak)	inactive
PLA_2[5]	80 (methyl esters)	>80	22	30	34

1. Micromolar concentration inhibiting 50% of enzyme activity.
2. Isolated from dog kidney.
3. Isolated from hog gastric mucosa.
4. 5-lipoxygenase activity measured in human polymorphonuclear leukocytes.
5. Phospholipase A_2 activity measured using heparinized human blood.
6. Not assayed.

CHAPTER III

UNPRECEDENTED OXYLIPINS FROM THE OREGON GREEN ALGA *ACROSIPHONIA COALITA*

ABSTRACT

The Oregon marine chlorophyte *Acrosiphonia coalita* contains unusual 18-carbon polyunsaturated fatty acids. A novel branched-chain conjugated trienal was discovered in which the aldehyde branches off of the fatty acid chain. A major secondary metabolite was characterized as an antimicrobial ten-carbon trienal. An alternative biogenetic pathway may produce novel linear trienones. A family of hydroxy-epoxy metabolites from *A. coalita* may represent modifications at the original sites of oxidation on the fatty acid chains. Selected compounds containing allylic alcohols were converted to benzoate derivatives and analyzed by circular dichroic spectroscopy to determine their absolute stereochemistries.

INTRODUCTION

This project began with the detection of a UV-active compound in crude extracts of the Oregon marine green alga *Acrosiphonia coalita* (Rupr.) Scagel, Garbary, Golden, *et* Hawkes. *A. coalita* belongs to the family Acrosiphoniaceae, order Ulotrichales, class Prasinophyceae of the division Chlorophyta.[243] In most of the older algal taxonomy texts, this alga is called *Spongomorpha coalita*.[244,245] According to current accepted taxonomy, *Acrosiphonia* cells are multinucleate, while *Spongomorpha* cells are uninucleate.[246]

A. coalita commonly occurs in the low- to mid-intertidal region of Oregon. Displaying an outward appearance of short, frayed pieces of green rope, it prefers a sandy substrate and quickly grows to abundance, in the absence of major scouring storms, by July. In Oregon, some specimens reach approximately 25 cm in length. Because *A. coalita* is an ephemeral alga, by mid-September, it starts to disappear from the coast of Oregon until the following spring. Other members of this group exhibit heteromorphic alternation of generations,[245,247,248] so it is likely that *A. coalita* is the gametophytic phase, while *"Codiolum petrocelidis"* Kuck. is the sporophytic phase of the algal life history. The sporophyte grows in the high- to mid-intertidal on rocks, and endophytically in the red alga *Petrocelis*, which appears as an insignificant, crustose slimy patch on rocks.

Recently, *A. coalita* was identified as a seaweed which the gumboot chiton avoids eating when presented with alternative algal species.[249] As part of an ongoing survey of the biomedicinal potential of northern Pacific North American seaweeds, *A. coalita* was identified early on as a candidate for further study. In antimicrobial bioassays, crude lipid extracts inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. Large-scale extraction and chromatography led to the isolation of a major UV-active compound, the 10-carbon, antimicrobial conjugated trienal which we have named "coalital". Although coalital was isolated from numerous collections, only one isolation sequence will be described. The more polar constituents of the organic extract were methylated and found to contain unusual polyunsaturated fatty acid

metabolites, exemplified by novel 18-carbon branched-chain trienals and straight-chain trienones which probably derive from stearidonic and α-linolenic acids. Hydroxy-epoxy octadecanoids were also isolated as methyl esters. We saw evidence for lipoxygenation in 9-*R* and 13-*S* positions, as revealed by the circular dichroism spectra of the semi-synthetic *p*-bromobenzoates.

This investigation is the first to discover unprecedented oxylipins in any marine or macrophytic green alga. An Italian group reported on the lipid composition of the terrestrial, acidophilic green alga *Dunaliella acidophila*.[250] After methylation of the acidic lipid extract, they isolated methyl (12*R*)-hydroxy-(9*Z*,13*E*,15*Z*)-octadecatrienoate, methyl (9*S*)-hydroxy-(10*E*,12*Z*,15*Z*)-octadecatrienoate, and methyl ricinoleate (from ricinoleic acid: (12*R*)-hydroxy-(9*Z*)-octadecenoic acid). These authors stated that the presence of hydroxy fatty acids in algae had not been reported before, although many papers on this subject, as mentioned in the introductory chapter, had appeared during the decade preceding the *D. acidophila* work.

Freshwater green algae have also demonstrated lipoxygenase activity. For example, *Chlorella pyrenoidosa* contains 9- and 13-lipoxygenase activity,[251] as well as hydroperoxide lyase activity.[143] Our research shows that *A. coalita* produces fatty acid metabolites similar to those of these two green algae, but surpasses these algae in the diversity of its metabolic pathways. Because *A. coalita* has motile gametes[252], it may have need of some as-yet undiscovered chemotactic substance to bring about the fusion of male and female gametes, as has been observed with hydrocarbon mating pheromones in the brown algae.[253]

RESULTS AND DISCUSSION

Acrosiphonia coalita was collected from Boiler Bay, Oregon, in July 1988, and immediately frozen in dry ice. Double extraction of the alga in 2:1 $CHCl_3$/MeOH yielded a crude organic extract which was subjected to vacuum chromatography[200-202] for initial fractionation. Those fractions eluting in 25-30% EtOAc in cyclohexane were repurified by a second vacuum chromatography. Fractions eluting from this second column contained UV-active compounds which did not react with CH_2N_2, and which were then purified on normal phase HPLC.

Conjugated-triene containing metabolites.

The major compound isolated (1) was optically active. Its IR spectrum showed a broad absorption at ν_{max} = 3400 cm^{-1}, which, in the absence of reactivity with CH_2N_2, was not attributable to a carboxylic acid. The UV spectrum showed an intense absorption at 312 nm, indicating an extensively conjugated π-electron system. Low resolution MS did not indicate any characteristic fragmentations, but did show $[M]^+$ *m/z* 166. By high resolution MS, the observed $[M]^+$ *m/z* 166.1001 (0.7 mmu dev.) gave a molecular formula of $C_{10}H_{14}O_2$, which contains four degrees of unsaturation. The ^{13}C NMR spectrum (Table III.1) contained one carbonyl and six olefinic resonances, accounting for all four degrees of unsaturation, indicating that 1 was an acyclic molecule.

The ^1H NMR spectrum (Table III.1) was well dispersed, first order, and understandable by conventional ^1H-^1H COSY analysis. Because no signal in the spectrum was coupled to more than two other signals, it was evident that the conjugated system consisted entirely of methines; i.e., there was no terminal olefin. An aldehyde doublet at δ9.56 was coupled to a doublet of doublets at δ6.17, which was in turn coupled by 15.3 Hz to a *trans*-oriented doublet of doublets at δ7.14. The conjugation continued from δ7.14 to a doublet of doublets at δ6.44, which was also coupled to a *trans*-oriented (14.5 Hz) doublet of doublets at δ6.67. Conjugation was extended from here to another doublet of doublets at

δ6.37, which was coupled to a *trans*-oriented (15 Hz) doublet of doublets at δ6.02. At this point, the conjugated system was coupled to a methinyl doublet of triplets at δ4.18, which was coupled to an ethyl terminus (δ1.61, 0.95). Thus, all the carbon-borne protons in the molecule were members of a single spin system.

Using one bond ^{1}H-^{13}C correlation spectroscopy, all of the carbon and proton resonances could be assigned, with the olefinic carbons displaying the typical pattern of alternation of shielding and deshielding seen with conjugated carbonyls. Furthermore, the methine proton at δ4.18 was borne on a carbon at δ73.38, indicating an attached oxygen, evidently a secondary alcohol, thus fulfilling the molecular formula and verifying the acyclic nature of the molecule, for which we propose the trivial name "coalital" (1).

Antimicrobial bioassays using the sensitivity disk method (see Experimental Section) showed that coalital (1) was effective at inhibiting the growth of the pathogenic yeast *Candida albicans* at doses as low as 100 μg/disk. One possible reason for this activity could be its similarity in chain length to undecylenic acid, a known antifungal agent. According to Paul and Fenical,[254] other possible reasons could include that the unsaturated aldehyde moiety is a strong electrophile which can react with proteins, possible resulting in their deactivation, and thus interfering with normal biological processes. Aldehydes may react with primary amines to form Schiff bases, thus possibly promoting protein denaturation. Biochemical nucleophiles such as hydroxyl and sulfhydryl groups may also add to the β-position of the unsaturated aldehyde in a Michael Addition.

Without any suitable model systems from the literature to compare with our optical rotatory dispersion data, we needed to pursue a different line of inquiry in order to ascertain the absolute stereochemistry of the secondary alcohol. We decided to proceed with modification of the hydroxyl position with a UV-chromophore which would enable us to apply Nakanishi's non-empirical exciton chirality method for absolute stereochemical determination.[255] Sharpless' and Nakanishi's groups were the first to apply the method to acyclic allylic alcohols.[256] This method was used in the stereochemical determination of the marine eicosanoids 8*R*-HETE and 8*R*-HEPE from the starfish *Patiria miniata*,[105]

as well as in the assignment of 12-*R* stereochemistry of two novel diol eicosanoids from *Farlowia mollis*.[121]

Because experience in our laboratory had shown that benzoylation reaction yields could be as low as 5%.[257], the first task was to isolate a sufficient amount of natural product starting material. In hindsight, the low yield may have been attributable to the use of solvents from which water had not been rigorously excluded, because water can hydrolyze acid chloride reagents. Another reason for low yields in the past is that material may have been lost during the aqueous workup step. A symmetrical (*para*-substituted) benzoyl chloride with a strong UV absorption was needed which would form a stable derivative. It was desirable that the UV absorption of the benzoate chromophore be relatively close to the natural product's chromophore, yet not so close that there could be any confusion as to which chromophore, the trienal or the benzoate, was responsible for which UV absorption. Therefore, we produced in 9% yield the 4-methoxybenzoate derivative (**2**), which showed a UV absorption at λ_{max} 258 nm in addition to the trienal chromophore at λ_{max} 310 nm. Acidic aqueous cupric acetate TLC spray reagent[227] proved indispensable in distinguishing the trienal and its derivative, which charred a characteristic yellow to grey-brown, from the acid chloride and its unwanted side-products, which did not char on TLC. Satisfactory ^{1}H NMR, FTIR, and mass spectra were recorded for derivative **2** and are reported in the experimental section.

In the circular dichroic spectroscopy (CD) analysis, we made the assumption, following Nakanishi's example,[256] that the preferred conformer or rotamer of derivative **2** in solution would be the one in which H-7 of the triene system and the α-benzoyl proton (H-8) are eclipsed, as depicted in the Newman projection in Figure III.2. Our assumption was substantiated by the observed 6.4 Hz coupling between H-7 and H-8, which, according to the vicinal Karplus correlation,[258] agrees with the proposed situation in which the dihedral angle between H-7 and H-8 is near 180°. A distinctive first Cotton effect (longer wavelength) and second Cotton effect (shorter wavelength) were observed in the CD spectrum of derivative **2**. These absorption phenomena result from the $\pi \rightarrow \pi^*$

electronic transitions of the trienal and the benzoate moieties, respectively. These transitions define vectors parallel to the long axis of each chromophore, resulting in a dihedral angle between the vectors.

In proceeding from the second to the first Cotton effect of the exciton split CD curve, if a positive slope is observed (i.e.: negative 2nd Cotton, positive 1st Cotton effects), then this is defined by convention as positive chirality. According to the theory, which was subsequently proven by numerous examples in practice,[256] we must trace through the smallest dihedral angle from the chromophore in front (nearest the observer) to the far chromophore (furthest from the observer), as shown in the Newman projection in Figure III.2. The orientation in which the Newman projection is drawn makes no difference; that is, no matter which end of the C-C bond connecting the two chromophores we make the observation, the relationship between the chromophores is the same, and will be either positive (clockwise) or negative (counterclockwise). In fact, a positive slope was observed in the CD spectrum, λ_{ext} 253, 284 nm ($\Delta\varepsilon$ = -6.6, +6.7), inferring positive chirality, and therefore, a clockwise relationship between the two chromophores. As shown in Figure III.2, the depicted absolute stereochemistry in the Newman projection allows us to define C-8 of derivative **2** as *S* and to assign *S* stereochemistry to natural product **1** as well.

While we were purifying sufficient amounts of coalital (**1**) for the benzoylation reaction, we noticed that a similar, less polar, UV-active compound (**3**) eluted from the HPLC column. Its optical rotation, UV, IR and ^{1}H NMR spectra were very similar to coalital (**1**). A comparison of the ^{1}H and ^{13}C NMR spectra for compounds **1** and **3** is tabulated in Table III.1. ^{1}H-^{1}H COSY analysis showed that except for some differences in chemical shifts and coupling constants, the connectivities of trienals **1** and **3** were the same. In fact, the only significant difference between trienals **1** and **3** was that in compound **3**, the central olefin of the triene moiety was of the *E* configuration by coupling constant analysis. The chemical shifts of the carbon atoms in compound **3** were assigned by a ^{1}H-^{13}C one-bond HETCOR experiment, for which the olefinic region is expanded in Figure III.3. The upfield chemical shifts of carbons 3, 4, 5, and 6 in isomer **3**

versus isomer **1** is entirely consistent with the more sterically compressed configuration of the *EZE* isomer, (**3**).[258,259]

Because the *EZE* configuration would be the predicted pattern resulting from two separate lipoxygenase reactions at opposite ends of three methylene-interrupted double bonds in a fatty acid[260-262] (see Figure III.6), we suspected that isomer **3** was the true natural product, and that its double bond isomer **1** may be an artifact of isolation. We had noticed that when isomer **3** was exposed to strong light during UV-detection on HPLC, acquisition of the UV spectrum, and during the optical rotatory dispersion measurements, it seemed that some of the material was being isomerized to the more thermodynamically stable isomer **1**. In order to prove this hypothesis, we purified unstable isomer **3** under low light conditions using RI detection. The compound was dissolved in $CDCl_3$, protected from light, and NMR spectra recorded. Next, the NMR tube and its contents were irradiated for one hour with 254 nm light. Afterwards, another NMR spectrum was then recorded on the same instrument. By inspection, the characteristic signals for both isomers **1** and **3** were present following irradiation. By integration of these signals, ca. 75% of isomer **3** had been converted to isomer **1** during the UV treatment. Exposure to high light conditions, either in the wild before collection, or in the laboratory during workup, may accentuate the conversion of "*iso*-coalital" (**3**) to the predominant coalital (**1**).

At this point, it was suspected that the lipid chemistry present in *A. coalita* might result from lipoxygenase acting on an ω-3 polyunsaturated fatty acid, followed by cleavage of the carbon chain, as depicted in Figure III.6. Based on our work with *Murrayella*,[208] described in the previous chapter, we thought that perhaps EPA might be the precursor to the *Acrosiphonia* trienals. We decided to investigate the more polar fractions of the original *A. coalita* vacuum chromatography (VC) in an attempt to locate longer-chain precursor molecules as their methyl esters. The original VC fraction which eluted in 40% EtOAc/cyclohexane contained compounds which by TLC analysis reacted with ethereal CH_2N_2. Therefore, the entire fraction was methylated with CH_2N_2 and subjected to a second VC followed by HPLC to yield a UV-active oil (**4**).

Because its UV spectrum (λ_{max} = 320 nm, ε = 37,000) was so similar in wavelength and intensity to those of trienals **1** and **3**, we suspected that compound **4** contained a similarly conjugated carbonyl. IR absorptions for compound **4** at ν = 3500 cm^{-1} (-OH) and at ν = 1671 and 1607 cm^{-1} (conjugated trienal) were also similar to those seen in the spectra of trienals **1** and **3**. In compound **4**, however, an additional absorption at ν = 1734 was attributed to a methyl-esterified carboxy terminus. Low resolution EI mass-spectra of compound **4** did not reveal any characteristic fragmentations, but did show $[M]^+$ *m/z* 320. By high resolution MS, the observed $[M]^+$ *m/z* 320.1987 gave a molecular formula of $C_{19}H_{28}O_4$, indicating six degrees of unsaturation in this methyl ester derivative of an 18-carbon fatty acid. Inspection of the ^{13}C NMR spectrum, which contained a total of 19 carbon resonances, showed that these unsaturations were attributable to four olefins, one ester carbonyl (δ174.31), and one aldehyde (δ193.91); hence, compound **4** was acyclic. The ^1H NMR spectrum showed three distinct termini, an aldehyde singlet (δ9.43), a methyl ester singlet (δ3.67), and an aliphatic methyl triplet (δ0.96), indicating that compound **4** was a branched-chain fatty acid derivative. Proton and carbon assignments appear in Table III.2.

The ^1H-^1H COSY plot of derivative **4** (Figure III.4) shows that the vicinal correlations are separated into two separate spin systems. Beginning with the triplet methylene at δ2.34, vicinal to a carbonyl, the first correlation is to a methylene multiplet at δ1.67, which is also coupled to a triplet of triplet methylene at δ1.43. This signal is further coupled to the doublet of triplets resonance of an allylic methylene at δ2.19, which couples to the olefinic methinyl doublet of triplets at δ5.38. *Cis*-coupling was apparent (J = 10.6 Hz) in the correlation of this olefin proton to its partner doublet of doublets at δ5.25, which was coupled in turn to a doublet methylene at δ3.11, marking the end of the first spin system.

The second spin system began with the methyl triplet at δ0.96, which was coupled to a methylene doublet of quartets at δ1.64. This methylene was further coupled to the doublet of triplet proton at δ4.19 of an oxygen-bearing methine. This was coupled to an olefinic methinyl doublet of doublets at δ6.02, which

showed 14.9 Hz *trans* coupling to a complex multiplet methine at δ6.43. This multiplet coupled to a 2H multiplet at δ6.66 which was coupled in turn to a one-proton multiplet (more complex than a broad doublet) at δ6.85, marking the end of that spin system. In the ^{1}H-^{13}C HETCOR experiment (Figure III.5) we saw that two distinct carbon resonances (δ141.15 and 127.05) underlay the two-proton second-order coupled signal at δ6.66. By the process of elimination, the quaternary olefin at δ140.55, in the absence of an ether linkage, bridged the gap between the between the two spin systems, connecting the doublet methylene at δ3.11 with the doublet olefin at δ6.85.

In order to prove this hypothesis, a series of nOe difference spectroscopy (nOeDIFF and nOeMULT) experiments was performed. First, in order to firmly place the aldehyde, rather than the ester, as being in conjugation with the triene in addition to its being a substituent of the lone quaternary olefinic carbon, we irradiated the aldehyde proton and saw enhancement only in the signal at δ6.85. Conversely, when we irradiated the multiplet at δ6.85, only the aldehyde singlet was enhanced. These results were interpreted as evidence that these two substituents are *cis*-oriented on a trisubstituted olefin. Furthermore, irradiation of the bis-allylic methylene doublet at δ3.11 enhanced the second-order coupled signal at δ6.66, attaching that methylene to the quaternary carbon in a *cis*-orientation to the proximate methine in the multiplet at δ6.66. Not only did these experiments unequivocally place the aldehyde in conjugation with the triene, as substantiated by the UV spectrum; but also, in accordance with priority rules, defined the stereochemistry of the trisubstituted olefin as *E*. By the process of elimination, and presupposing biogenesis from stearidonate (the 18:4 ω-3 polyunsaturated fatty acid, see Figure III.6), the methyl ester must be placed vicinal to the triplet methylene at δ2.34.

At this point, the geometry of all olefins in compound 4 had been established except for the middle olefin of the conjugated triene. Because of the second-order coupling and coincident chemical shifts of the two protons in question, conventional coupling constant analysis was out of the question. In the absence of firm nOe, J-resolved, phase-sensitive double quantum filtered COSY,

or PANIC spectral simulation results, all of which were attempted, we assigned this double bond geometry by comparison of NMR chemical shift assignments for branched-chain trienal **4** (Table III.2) with the NMR data for *EEE* trienal **1** and *EZE* trienal **3**. The olefin geometries of trienals **1** and **3** were determined by straightforward coupling constant analysis (Table III.1). Chemical shifts for all three compounds are reported in the same solvent, $CDCl_3$, in Tables III.1 and III.2.

Beginning with the position β to the aldehyde carbonyl in the trienal system, the proton chemical shift for derivative **4** (δ6.86) is closer to that of *EEE* trienal **1** (δ7.14) than that of *EZE* trienal **3** (δ7.61). At the γ-position, derivative **4** (δ6.66) is more in agreement with trienal **1** (δ6.44) than with trienal **3** (δ6.23). The δ-position of derivative **4**, with respect to both ^1H (δ6.66) and ^{13}C chemical shift (δ141.15), agreed better with trienal **1** (δ6.67, 142.01) than with its double-bond isomer **3** (δ6.44, 138.34). Finally, at the crucial ε-position, we see a much better agreement of compound **4** (δ6.45, 129.36) with *EEE* trienal **1** (δ6.36, 129.06) than with *EZE* trienal **3** (δ6.85, 124.16), which contains a sterically compressed, deshielded carbon in this position. Further evidence for the *EEE* geometry in branched-chain trienal **4** comes from the fact that despite many manipulations in the laboratory, we saw no evidence whatsoever of isomerization as could occur in an unstable *EZE* arrangement.

In the mass spectrum of the TMSi-ether of Me-ester **4**, we observed a significant fragmentation at *m/z* 131 $[TMSiOCHEt]^+$, giving further proof that the compound was hydroxylated at the ω-3 position. In oxylipin biosynthesis, ω-3 lipoxygenation is an uncommon phenomenon. Powell and Gravelle reported that fetal calf aortal enzyme preparations converted EPA to 18-HEPE of undefined stereochemistry,[263] the only documented report of 18-HEPE thus far. More recently, Oliw reported 18-*R* hydroxylation of arachidonate (AA) by monkey seminal vesicles;[264] however, this was not a lipoxygenase-type reaction, as there was no double bond present in the AA substrate at the position of oxygenation; and so, consequently, no conjugated diene was formed. Soon after, Powell and Gravelle reported that LTB_4 may be hydroxylated at the 18-position during

metabolism by PMNs,[265] but again, for the same reasons as above, this is not a lipoxygenase-mediated reaction. A Korean group has reported that when α-linolenate was incubated with soybean lipoxygenase, four isomers of 9,16-dihydroperoxy-10,12,14-octadecatrienoic acids were isolated.[266] Based on UV and MS data, these were the 9*S* *EZE* and *EEE* isomers, both diastereomeric at C-16. They performed a chemically catalyzed isomerization of the *EZE* to the *EEE* isomer, although confirmatory NMR data was absent from their report. Considering the facile photoisomerization we performed on trienal **3** from *Acrosiphonia*, it seems likely that the isomerization of the soybean-derived product was successful as well.

Quite recently, a Russian group, using a potato lipoxygenase preparation and α-linolenate, was able to isolate enough (9*S*),16-dihydroxy-(10*E*,12*Z*,14*E*)-octadecatrienoate to report ^{1}H NMR data, unequivocally assigning the double bond configurations.[267] Particularly exciting in this report is mention of an unidentified compound, to be published separately, with a UV λ_{max} = 309 nm, extremely close to that of trienals **1** and **3**, and to the *Acrosiphonia* trienones described below. The apparent similarity between fatty acid metabolism in the potato and *Acrosiphonia* makes it likely that the Russian group will soon be reporting on compound(s) very similar or identical to those reported in this chapter. Branched, α,β-unsaturated aldehydes are unprecedented in the fatty acid class; however, the freshwater green alga *Botryococcus braunii* produces non-isoprenoid long-chain hydrocarbons containing this functional group.[268,269]

Having defined branched-chain trienal **4** as methyl-9-formyl-15-hydroxy-(6*Z*,9*E*,11*E*,13*E*)-heptadecatetraenoate, all that remained to the structure elucidation was stereochemical assignment at the ω-3 position. The optical rotatory dispersion data (see Experimental Section) was of extremely low magnitude in comparison to trienals **1** and **3**, evidence that compound **4** was racemic. A similar lack of optical activity was noted in the marine eicosanoid ptilodene,[118] which also contained an allylic alcohol vicinal to a conjugated carbonyl-containing olefin system, although the chromophores are not the same. In order to ascertain the stereochemistry of compound **4**, its methoxamine

derivative was prepared and treated with ozone.[270] A menthoxychlorocarbonate derivative of the ozonized fragment obtained from the ω-end of the fatty acid chain was prepared and analyed by GC and GCMS versus standards, revealing that the molecule was racemic at the ω-3 position. In consideration of the fact that trienals **1** and **3** and the analogous trienones (described below) are all optically active, perhaps compound **4** is stereospecifically oxygenated, but at an easily epimerizable site. Alternatively, it could be formed non-enzymatically from an unstable enzyme product.

During the HPLC purification of compound **4**, a less-polar peak was collected as a shoulder yielding an oil (**5**) following further purification by HPLC . UV and ^{1}H NMR analysis (Table III.2) showed that compound **5** was the 6,7-dihydro analog of compound **4**, which would logically result from the substitution of α-linolenate for stearidonate in the proposed biosynthetic manifold (Figure III.6). Proton and double bond assignments for compound **5** followed from analogy to branched-chain trienal **4**. During this purification, 0.6 mg of an even less polar metabolite (**6**) was recovered.

Although the UV spectrum of compound **6** (λ_{max} = 314 nm) was more reminiscent of trienals **1** and **3** than of branched-chain trienal **4**, its ^{1}H NMR spectrum (Table III.3) lacked an aldehyde resonance altogether. ^{1}H-^{1}H COSY analysis showed two separate spin systems similar to those of compound **4**. Hydroxylation at the ω-3 position was apparent, but in this compound there was a definite *cis* coupling (11.3 Hz) at the middle triene olefin, while the two flanking olefins were *trans* (15, 15.3 Hz). Unlike compound **4**, compound **6** contained six olefinic protons, with an additional 15.3 Hz doublet appearing at δ6.18, virtually the same chemical shift as the α-position of trienals **1** and **3**.

The other spin system of compound **6** consisted of aliphatic multiplets bounded at each end by characteristic α-carbonyl triplets. We presumed that the higher-field triplet at δ2.30 was vicinal to the methyl ester. Based on the apparent pattern of C-9 oxidation in this alga, that left the triplet at δ2.57 vicinal to the ketone at C-9. Further evidence for structure **6** was found in the low resolution chemical ionization mass-spectrum of its TMSi-ether derivative. The observed

$[M + H]^+$ *m/z* 395 of the TMSi-ether derivative corresponded to a calculated molecular formula of $C_{19}H_{30}O_4$ for non-silylated compound **6**. The fragment at *m/z* 185 $[MeOCO(CH_2)_7CO]^+$ represents the expected α-cleavage between the C-9 ketone and the olefin at C-10, as was also observed in the mass spectrum of methyl 9-oxo-10,12,15-octadecatrienoate.[138] The more stable *EEE* isomer, (**8**), of *EZE* trienone **6**, for which a more complete data set was obtained, was isolated later in this project.

A less-polar original VC fraction than that which contained compounds **4-6** was methylated with CH_2N_2 and rechromatographed in the vacuum mode over silica gel. One subfraction contained a UV-active compound and was further purified by repetitive HPLC to yield an oil (**7**). FTIR showed no hydroxyl, but did predict an ester (ν_{max} = 1736 cm^{-1}) as well as a triene conjugated to a carbonyl (ν_{max} = 1672, 1615 cm^{-1}). The UV spectrum (λ_{max} = 324 nm) was closer in frequency to branched chain-trienals **4** and **5** than to compounds **1**, **3**, or **6**. In addition to $[M]^+$ *m/z* 318, the LR electron impact mass-spectrum displayed a fragmentation at *m/z* 261, which could be interpreted as $[M - COEt]^+$. In the high resolution EIMS, we observed $[M]^+$ *m/z* 318.183 which corresponds to a molecular formula of $C_{19}H_{26}O_4$, thus indicating seven degrees of unsaturation in the methyl ester derivative.

The ^1H NMR spectrum of compound **7** (Table III.2) was similar to that for branched-chain trienal **4**; however, no α-hydroxyl resonance was present. The entire spectrum was first-order in this case, however, and by ^1H-^1H COSY analysis, divided into three distinct spin systems. The first of these was simply an isolated ethyl group vicinal to a carbonyl, as substantiated by the mass-spectral fragmentation data (see Experimental). The other end of the molecule was practically identical with respect to chemical shift and coupling constant analysis to branched-chain trienal **4** from H-2 through H-8.

Based on the close agreement of UV, IR, and ^1H-NMR data for compound **7** to compound **4**, the aldehyde is designated as a substituent of C-9, and placed *trans* to C-11 as in compound **4**. We have assigned the remaining proton signals to the conjugated triene moiety, based on chemical shift comparisons to

compounds **4** and **5** (Table III.2). Coupling constant analysis shows that both disubstituted olefins in this system are of the *E*-geometry. The trisubstituted olefin is assumed to be *E* as well on the basis of these comparisons. Due to the additional ketone in conjugation with the triene system of compound **7** versus compounds **4** and **5**, an increased polarization is apparent in the chemical shifts of the triene protons. It is surprising that the UV-spectrum of compound **7** is so similar to compounds **4** and **5**, considering the additional conjugated carbonyl in the chromophore of **7**.

The last triene-containing compound isolated from *A. coalita* came from the most polar fractions of the original VC, which were methylated with CH_2N_2 and rechromatographed. UV-active metabolite containing fractions were repurified on HPLC to yield an oil (**8**). FTIR showed a broad OH stretch (ν_{max} = 3340), an ester (ν_{max} = 1742), and a conjugated carbonyl (ν_{max} = 1678). The UV spectrum (λ_{max} = 312) was reminiscent of either a conjugated trienal or trienone. Low resolution EIMS showed $[M]^+$ *m/z* 322, as well as the same fragmentation at *m/z* 185 seen for trienone **6**. In the high resolution EIMS, we observed $[M]^+$ *m/z* 322.214, which corresponds to a molecular formula for the methyl ester derivative of $C_{19}H_{30}O_4$ and contains five degrees of unsaturation. The ^1H NMR spectrum (Table III.3) was similar to that of *EZE* trienone **6**, except for the triene portion, which by coupling constant analysis was in the *EEE* orientation. The ^{13}C NMR spectrum showed that the degrees of unsaturation were accounted for by the three olefins of a triene, an ester carbonyl, and the C-9 ketone at δ200.67. ^1H-^1H COSY and ^1H-^{13}C HETCOR experiments gave the proton and carbon assignments listed in Table III.3.

The optical rotatory dispersion curve of compound **8** was of the same sign and magnitude as that of coalital (**1**) (see Experimental section for details), assigning *S* stereochemistry at C-16. Evidence for the optical purity of *EZE* trienone **8** was found using a chiral HPLC column, from which compound **8** eluted as a single peak. However, it was reported that with this type of column, which is made of silica derivatized with (*R*)-(-)-N-3,5-dinitrobenzoylphenylglycine (DNBPG), variation in resolution between columns may be observed.[271]

Based on our experience with the unstable *EZE* trienal **3**, we attempted to isomerize *EZE* trienone **6** using the same procedure. ^{1}H NMR spectra of compound **6** taken in $CDCl_3$ before and after a one hour irradiation with 254 nm light clearly show proton resonances of trienone **8** appearing, as the resonances of isomer **6** diminish in intensity, following UV irradiation. Approximately 80% of trienone **6** isomerized to trienone **8** during the experiment.

Biogenetic origins of triene metabolites.

In order to obtain conjugated triene metabolites from *Acrosiphonia*, enzymatic and/or non-enzymatic processes may be at work (Figure III.6). In a lipoxygenase-type reaction sequence, proton abstraction at C-11 and C-14 bis-allylic sites, followed by insertion of molecular oxygen at C-9 and C-16 would give the 9,16 dihydroperoxide shown. Glutathione peroxidase-type activity[272] in the alga could then reduce the hydroperoxide(s) to secondary alcohol(s), which might then be oxidized to the ketone non-enzymatically or by some type of oxidase activity. In a recent study,[273] conversion of 12-hydroperoxyeicosatetraenoate (12-HPETE) directly to the 12-oxo oxidation state was catalyzed enzymatically and non-enzymatically by a horseradish peroxidase, cytochrome c, boiled platelets, and hemoglobin. Such activities could have parallels in the plant kingdom, where cytochromes and iron porphyrins are well-known.

A possible route to the chain-cleaved coalitals **1** and **3** can be visualized in analogy to an aldehydic product of AA lipoxygenation followed by chain cleavage, 12-oxo-(5*Z*,8*E*,10*E*)-dodecatrienoate, which was first isolated from the red alga *Laurencia hybrida*.[195] Brash et al. later demonstrated that the 8*Z* isomer was formed when excess arachidonate, 12-HPETE, and the purified 12-lipoxygenase enzyme from porcine leukocytes were incubated under anaerobic conditions.[274] Using a blood platelet homogenate, it has been demonstrated that anaerobic conditions are not necessary for non-enzymatic production by hematin of 12-oxo-eicosatetraenoate or of either 8*E* or 8*Z* isomer of the 12-oxo chain cleavage product.[275] Obviously, biosynthetic experiments are necessary to determine the

mechanism(s) of chain-cleaved aldehyde formation and other transformations in *A. coalita*. A related aldehydic product of hydroperoxide lyase in terrestrial plants, 12-oxo-(10*E*)-dodecenoate, has been identified as "traumatin", a wound hormone.[143] The chain-cleaved coalitals **1** and **3** could also result from the action of hydroperoxide lyase[143,212] on a 9-OOH substrate, but unconventionally cleaving the 8-9 instead of the 9-10 bond.

In the literature, evidence has been mounting for allene oxide intermediates in marine invertebrate[50,51,72-74,276] and terrestrial plant oxylipin biosynthesis.[148-150,277-278] As shown in Figure III.6, we can envision abstraction of H-10 of the 9-hydroperoxy intermediate, catalyzed by an allene oxide synthetase (hydroperoxide dehydrase) activity, to give the allene oxide intermediate. Collapse of the epoxide to form the carbonyl at C-9 could provide the driving force for the migration of the C-8,9 bond to C-10, resulting in branched-chain trienals **4**, **5**, and **7**. Alternatively, H-9 of the allene oxide intermediate could be abstracted, allowing its bond to form the ketone at C-9, resulting in the conjugated trienone arrangement as seen in compounds **6** and **8**.

A final alternative for branched chain trienal biogenesis can be envisioned in the continuation of Figure III.6. Following the formation of the 9-hydroperoxy intermediate, displacement of hydroxide by the π-electrons of the C-10,11 olefin forms an oxetane ring, leaving a cation at C-10. Migration of the C-8,9 single bond to C-10 forms a branched carbon chain and places the cation on the protruding carbon. Migration of the C-11 oxygen bond to the protruding carbon places the cation on what was formerly C-11 of the linear fatty acid chain. Proton abstraction at the branchpoint forms the final olefin in the trienal chromophore.

Hydroxy-epoxy metabolites.

During the process of purifying some of the UV-active metabolites from *Acrosiphonia*, we noticed that many were contaminated with UV-inactive materials which charred blue with aqueous acidic cupric acetate, reminiscent of the hepoxilins described in the previous chapter. Sub-fractions of the methylated

material which contained branched-chain trienal **4** displayed the characteristic ^1H NMR signals associated with the hydroxy-epoxy moiety (as discussed in the previous chapter). Repeated attempts to purify the major compound on HPLC with RI detection were unsuccessful, even when employing HPLC methods specifically developed for hepoxilins from the literature.[228,231] The material which we eventually characterized by ^1H and ^{13}C NMR (see Table III.4) was an approximate 4:1 mixture of two very similar compounds. Both the ^1H and ^{13}C data sets were extremely close matches to those reported for methyl (12*S*,13*S*)-epoxy-(11*R*)-hydroxy-(9*Z*,15*Z*)-octadecadienoate (**9**), which had previously been reported from rice plants which were infected with the rice blast fungal disease.[236] Because of the agreement of our ^1H and ^{13}C chemical shifts and coupling constants with the published values, we were certain that we had isolated the *threo* diastereomer and not the *erythro*, for which data was also published in the rice blast fatty acid report.[236]

In order to verify the position of the hydroxyl group, we silylated compound **9** for GCMS analysis. In the mass spectrum, the fragment at *m/z* 285 $[TMSiOCHCH=CH(CH_2)_7COOMe]^+$ strongly supported hydroxyl placement at C-11. This and other fragmentations were comparable to published values for the same derivative.[279] This compound, along with its *erythro* isomer, was identified as a major product of 13-hydroperoxyoctadecadienoate decomposition by hemoglobin[280] and hematin.[281]

With firm evidence for the relative stereochemistry of compound **9**, it seemed possible to solve its absolute stereochemistry by comparison of its optical rotatory properties with the literature values.[236] Although the Japanese researchers did not report the optical rotation of the underivatized methyl ester, they did report the optical rotation of its *para*-bromobenzoate ester, which was made in order to apply Nakanishi's CD method for stereochemical determination of acyclic allylic alcohols.[256] We produced *para*-bromobenzoate ester **10** in a similar fashion to derivative **2**, except for the use of a different acid chloride. Details and analytical data appear in the Experimental section. The optical rotation of our derivative **10** ($[\alpha]_D$ +41°) was dextrorotatory, as was the literature

value (+26°).[236] We felt that the difference in magnitude was significant enough to warrant that a CD spectrum be acquired for derivative **10** as well. The spectrum which we recorded ($\Delta\varepsilon$ = +7.74, λ_{max} 245 nm) was in agreement with the literature value for derivative **10**[236] ($\Delta\varepsilon$ = +5.94, λ_{max} 244 nm).

As in the analysis of derivative **2**, we made the assumption that the preferred rotamer of derivative **10** is the one in which the olefin is eclipsed by the allylic α-benzoyl proton (H-11),[256] as depicted in the Newman projection in Figure III.7. The positive sign of the first Cotton effect at 245 nm in the CD spectrum of derivative **10** indicates positive chirality, and therefore, a clockwise direction of movement going from the chromophore in front to the chromophore behind, as shown. According to Nakanishi, since the 200 nm region is perturbed and overlaid by other transitions, the sign of the non-conjugated olefin's Cotton effect is not diagnostic.[256] Inspection of the Newman projection shows that this orientation results in *R* stereochemistry at C-11.

After benzoylation of the mixture which had yielded predominantly derivative **10**, it was noticed that this derivative could be easily separated from its "contaminant" by HPLC; and that, just as the Japanese group had discovered,[236] the contaminating substance was the 15,16-dihydro homolog of compound **9**, isolated as the benzoate derivative **11**. In the mass-spectrum of derivative **11**, a characteristic double $[M]^+$ *m/z* 508 and 510, resulting from the two isotopes of bromine in the synthetic benzoate ester portion, dictated a molecular formula of $C_{19}H_{34}O_4$ for the non-benzoylated methyl ester derivative, resulting in three degrees of unsaturation. Fragmentations at *m/z* 439 and 437 $[M - (CH_2)_4CH_3]^+$ indicated that derivative **11** was oxidized at the ω-6 position, as was compound **10**.

The IR spectrum of compound **11** showed an ester carbonyl absorbance at ν_{max} = 1723 cm^{-1}, while the ^1H NMR spectrum, which was very similar to that of derivative **10** except for having one less olefin, showed two *trans*-coupled epoxide protons (δ3.01, 2.90) and two *cis*-coupled olefin protons (δ5.71, 5.49), thus accounting for all three degrees of unsaturation attributable to the native, underivatized fatty acid portion of the molecule. The 6.1 Hz coupling constant between the α-benzoyl proton at δ5.58 (H-11) and the epoxide proton at δ3.01 (H-

12) was evidence for a *threo* relationship between the benzoate ester and the epoxide oxygen.[155, 233-235] The large 9.3 Hz coupling between the H-10 olefin and H-11 substantiated the argument for an eclipsed conformation in the preferred rotamer.[256]

The optical rotation, UV, and CD data for derivative **11** were very close to those reported for derivative **10**, the only difference between the two compounds being the lack of the ω-3 olefin in derivative **11**. The Newman projection in Figure III.7 and the stereochemical analysis for derivative **10** are applicable to derivative **11** as well, thus giving an *R* configuration at C-11. Because the ester and the epoxide oxygens are *threo* to each other, it follows that the stereochemistry at C-12 and C-13 must be *S*, as in derivative **10**.

An optically active compound (**12**) was isolated in a similar fashion to methyl ester **9** from neighboring chromatography fractions (see Experimental section for details). The IR spectrum included a broad -OH stretch (ν_{max} = 3420 cm^{-1}) and a strong absorption attributable to an ester carbonyl (ν_{max} = 1740 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of compound **12** (see Table III.4) were reminiscent of compound **9**, except that compound **12** had one less double bond. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR experiments conclusively proved that the same partial structure from compound **9**, an allylic secondary alcohol vicinal to a *trans* epoxide, was also contained in compound **12**. The placement of this partial structure within the carbon chain could not be approached by conventional NMR studies due to the plethora of overlapping aliphatic resonances. GCMS analysis of the TMSi-ether of compound **12** showed a prominent fragmentation at *m/z* 199 $[TMSiOCHCH{=}CH(CH_2)_4CH_3]^+$, which by comparison to literature data,[235,279,281] located the hydroxyl at C-11, and therefore the epoxide at C-9 and -10.

Analysis of the coupling constants in the ^{1}H NMR spectrum (Table III.4) indicated that, as in compound **9**, the epoxide oxygen was *threo* to the allylic hydroxyl oxygen. Given this relative stereochemistry, the absolute stereochemistry was approached in the same fashion as for compound **9**. The *p*-bromobenzoate derivative (**13**) was produced for CD analysis (see Experimental section for details). Along with other analytical data, the ^{1}H NMR spectrum of derivative **13**

was satisfactory and showed a 9.3 Hz coupling between H-11 and H-12, thus giving the preferred eclipsed rotamer as depicted in the Newman projection in Figure III.8. The negative sign of the first Cotton effect in the CD spectrum indicates negative chirality between the benzoate at C-11 and the olefin at C-12, resulting in the *S* stereochemistry as shown. Verification of the molecular weight of compound **12** was found in the MS analysis of derivative **13**, which showed a double $[M]^+$ *m/z* 510 and 508, which calculates to a molecular formula of $C_{19}H_{34}O_4$ for non-benzoylated compound **12**. Therefore, the algal natural product is (9*R*,10*R*)-epoxy-(11*S*)-hydroxy-(12*Z*)-octadecenoate.

The last and most polar compound (**14**) in this structure class to be isolated from *A. coalita* was purified in a similar fashion to compounds **9** and **12**. The IR spectrum of methyl ester **14** was practically indistinguishable from that of compound **12**. In many ways, the ^1H NMR spectrum of compound **14** (see Table III.4) was very similar to the spectra of compounds **9** and **12**. However, due to fortuitous non-overlap within the olefinic region, a ^1H-^1H COSY experiment (Figure III.9) gave considerable structural information. The terminal methyl triplet at δ0.98 was coupled to an allylic methylene doublet of quartets at δ2.06, which was further coupled to a *cis* olefin (J = 10.7 Hz). This ω-3 olefin was also coupled to a bis-allylic methylene doublet of doublets at δ2.85, which was also coupled to an ω-6 *cis* olefin. The ω-6 olefin was further coupled to an α-hydroxyl methinyl doublet of doublets of doublets at δ4.33, which was coupled in a *threo* fashion to a vicinal epoxide doublet of doublets at δ2.80. A *trans* coupling (2.3 Hz) was measured between this proton and its partner on the epoxide ring, a methinyl doublet of triplets at δ2.93. From this point on, the spin system was coupled into overlapped aliphatic multiplets and could not be traced further.

However, GCMS analysis of the TMSi-ether derivative of compound **14** showed, in addition to $[M - CH_3]^+$ at *m/z* 381, a characteristic α-cleavage at *m/z* 197, similar to the fragment seen at *m/z* 199 in the TMSi-ether of compound **12**, but containing an additional olefin at the ω-3 position. Compound **14** is therefore the ω-3 homolog of compound **12**. Having shown that these two compounds possess the same relative stereochemistry, the virtual coincidence of their optical

rotations at two different wavelengths (see Experimental section) assigns to compound **14** the same absolute stereochemistry at all three stereocenters as compound **12**. Therefore, compound **14** is methyl (9*R*,10*R*)-epoxy-(11*S*)-hydroxy-(12*Z*,15*Z*)-octadecadienoate.

Biogenesis and possible role of hydroxy-epoxy metabolites.

A proposed biosynthesis of hydroxy-epoxy metabolites in *A. coalita* is depicted in Figure III.10. Based on the limited scope of this research, it appears that ω-6 linoleate and ω-3 α-linolenate are the main substrates for 9- and 13-lipoxygenation. It has been shown with soybean lipoxygenase that the same enzyme can oxidize either C-9 or C-13.[282] The fact that hydroxy-epoxy compounds were isolated is evidence that lipoxygenase-type enzyme(s) produce(s) the 9- and 13-hydroperoxide intermediates shown. Following the formation of these unstable intermediates, an oxygen-rebound mechanism[237,281] could be invoked for the elaboration of the hydroxy-epoxy products. It is noteworthy that monohydroxy oxylipins were not isolated from this alga. Conceivably they were overlooked, or perhaps most of the hydroperoxide intermediates which are formed in the alga are consumed by biosynthetic processes which do not lead to monohydroxy products. Note also that while an approximately 1:1 mixture of *erythro/threo* hepoxilin B diastereomers were isolated from the red alga *Murrayella periclados* (see previous chapter), only *threo* isomers were recovered from *A. coalita*. This finding is evidence that the transformations of the hydroperoxide intermediates to the hydroxy-epoxy products may be catalyzed by the lipoxygenase enzyme(s) itself in *Acrosiphonia*, a mechanism which, in the case of the soybean lipoxygenase-1, resulted in the formation of a strictly *threo* product.[235] A non-enzymatic rearrangement catalyzed by an iron porphyrin would lead to a 50:50 mixture of *erythro* and *threo* products, as discussed in the previous chapter.

In the rice plant infected with the fungus *Pyricularia oryzae*, the *threo* free acid of compound **9** predominated over the *erythro* diastereomer in a 13:5 ratio.[236] Normally, rice seedlings exhibit both 9- and 13-lipoxygenase activities.[283] These

oxidase activities increase when the rice plant becomes infected with the fungus.[284] Oxidized fatty acid metabolites from these pathways inhibit the growth of the rice blast fungus in biological assays.[284,285] Perhaps the oxidized fatty acid metabolites of *A. coalita* are produced in response to microbial infestation as well.

Throughout the plant kingdom, 9- and 13-lipoxygenase activities are widely distributed.[135] Early in the development of this field of research, Hamberg demonstrated that the stereospecificity of oxygen insertion resulting from 9- and 13-lipoxygenase activity in corn and soybeans varied according to source of enzyme and experimental conditions.[286] More recently, Funk's group, using soybean lipoxygenase-1, surmised that *S* oxygen insertion by the enzyme at both C-9 and C-13 of linoleate predominated in a 4:1 ratio over the *R*-enantiomer.[287] Much more research would be necessary to determine the different types of lipoxygenase activities in *A. coalita*, and to determine why 9-*R* and 13-*S* products predominate, and whether their formation could be initiated by the same enzyme, as postulated in Figure III.11. The role of the 16-*S* oxygenase activity involved in conjugated triene formation in this alga, and its relationship to the putative 9- and 13-lipoxygenase activities, is also unclear at this time.

EXPERIMENTAL

UV spectra were recorded on Beckman DB-GT and Hewlett Packard (HP) 8452A Diode-array spectrophotometers. IR spectra were recorded on a Nicolet 510 FT-IR spectrophotometer. Optical rotations and ORD data were obtained using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on Bruker AM 400 spectrometer operating at a frequency of 400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C spectra, or on a Bruker ACP 300 spectrometer operating at frequency of 300.13 MHz for ^{1H} and 75.47 MHz for ^{13}C acquisitions. All chemical shifts are reported relative to TMS internal standard. Coupling constants are reported in Hz and are accurate to within 0.5 Hz. LR EIMS data were obtained on a Finnigan 4023 spectrometer with both direct probe and GC inlet capabilities, a Varian MAT CH7 with direct probe inlet, or on a HP 5970B mass selective detector connected to a HP 5890 GC. HR EIMS was run on a Kratos MS 50 TC. Gas-liquid chromatography was performed using a HP 5890 GC equipped with a methylsilicone capillary column (HP Ultra-1, 25 m, 0.33 μm thickness) and helium carrier gas. Circular dichroism (CD) was measured on JASCO J-41A and J-10 spectro-polarimeters. HPLC utilized Waters M6000A and M45 pumps, R401 differential refractometer and Lambda-Max 480 Variable Wavelength detectors. Waters U6K and Rheodyne 7125 HPLC injectors were employed. TLC utilized Merck aluminum-backed TLC sheets with fluorescent indicator (silica gel F_{254}). Chromatography solvents were distilled from glass prior to use. All liquid chromatography was performed in the normal phase mode.

Collection, extraction, and chromatography. The intertidal green alga *Acrosiphonia coalita* (Rupr.) Scagel, Garbary, Golden, *et* Hawkes (entire thalli, 3 gal. fresh volume, 682 g dry wt.) was collected in July 1988 at Boiler Bay, Oregon, and immediately frozen in dry ice. A voucher specimen has been deposited at the Department of Botany and Plant Pathology Herbarium at Oregon State University. The collection was defrosted overnight in distilled water at 4°C,[112] after which, the water was decanted and the algae extracted 2X with 2:1 $CHCl_3$/MeOH, yielding 6.89 g of dark green crude extract. This extract was

vacuum chromatographed with a step-gradient of EtOAc in cyclohexane. Fractions which eluted in 25-30% EtOAc in cyclohexane (299 mg) were further purified via a second VC using the same solvents. Fractions eluting from this column in 15% EtOAc in cyclohexane (129 mg) were enriched in UV-active compounds, and were purified on HPLC (300 nm detection, Alltech Rsil 10 μm, 1 x 50 cm, 5% iPrOH in hexanes). The major UV-active compound (13.4 mg, 0.19% yield) was collected and characterized.

(8S)-hydroxy-(2E,4E,6E)-decatrienal (1). FTIR ν_{max}^{film} cm^{-1}: 3400 (br OH), 2966, 2932, 1673, 1612, 1163, 1126, 1015, 996. UV λ_{max}^{MeOH} nm: 312 (ε = 43,000). ORD: $[\alpha]_D^{25}$ +21°, $[\alpha]_{578}^{25}$ +22°, $[\alpha]_{546}^{25}$ +25°, (*c* 0.63, acetone). LR EIMS (probe) 70 eV *m/z* (rel. int.): 166 $[M]^+$ (31), 137 (22), 109 (100), 94 (58), 81 (85), 79 (55), 57 (48). HR EIMS *m/z* obs. $[M]^+$ 166.1001 ($C_{10}H_{14}O_2$, 0.68 mmu dev.) For ^1H and ^{13}C NMR data, see Table III.1.

(8S)-(4-methoxybenzoyl)-(2E,4E,6E)-decatrienal (2). Trienal 1 (8.4 mg) was dissolved in 2 ml dry CH_2Cl_2. To this solution was added 2 ml triethylamine, 44 μl (6 equiv.) of 4-methoxybenzoyl chloride, and a catalytic amount of *p*-dimethylaminopyridine (DMAP). The reaction mixture was refluxed for four hours, after which the solvents were removed in vacuo. The dried reaction product was triturated with cyclohexane for application to a vacuum chromatography column. Several nonpolar fractions (45 mg) from the EtOAc/cyclohexane step gradient were deemed by TLC to be of sufficient purity for injection on HPLC (257 nm detection, Waters μPorasil 10 μm, 2 x [3.9 x 300 mm], 7 % EtOAc in hexanes). This was followed by additional HPLC (311 nm detection, columns as previous, 2% iPrOH in hexanes) to yield pure benzoate derivative 2 (1.4 mg, 9% yield). FTIR ν_{max}^{film} cm^{-1}: 2969, 2933, 1708, 1679, 1607, 1511, 1257, 1168, 1102, 1026, 849, 770. UV λ_{max}^{MeOH} nm: 209, 258, 310 (ε = 17,000; 26,000; 43,000). CD (MeOH): $\Delta\varepsilon$ = -6.6, +6.7 (λ_{max} 253, 284 nm). $[\alpha]_D^{23}$ +81° (*c* 0.13, acetone). ^1H NMR (300 MHZ, $CDCl_3$): 9.56 (1H, *d*, J = 7.9 Hz, H-1), 8.02 (2H, *d*, J = 9.0 Hz), 7.12 (1H, *dd*, J = 15.2, 11.2 Hz, H-3) 6.94 (2H, *d*, J = 9.0 Hz), 6.66 (1H, *dd*, J = 14.9, 10.7 Hz, H-5), 6.46 (1H, *dd*, J = 14.9, 11.2 Hz, H-4), 6.41 (1H, *dd*, J = 15.2, 10.7 Hz, H-6), 6.16 (1H, *dd*, J = 15.2, 7.9 Hz, H-2), 6.02

(1H, *dd*, J = 15.2, 6.4 Hz, H-7), 5.51 (1H, *dt*, J = 6.4, 5.9 Hz, H-8), 3.87 (3H, *s*, -OMe), 1.82 (2H, *m*, H-9), 1.00 (3H, *t*, J = 7.6 Hz, H-10). LR EIMS (probe) 70 eV *m/z* (rel. int.): 300 $[M]^+$ (7), 148 (3), 135 (100), 107 (6), 94 (20), 77 (25).

(8S)-hydroxy-(2E,4Z,6E)-decatrienal (3). A less-polar UV-active entity was detected during the HPLC purification of trienal 1. This material was purified using the same conditions as for 1, but in a semi-darkened laboratory with RI detection. FTIR ν_{max}^{film} cm^{-1}: 3400 (br -OH), 2966, 2931, 1673, 1609, 1163, 1124, 1012, 999, 971. UV λ_{max}^{MeOH} nm: 313 (ε = 31,000). ORD: $[\alpha]_D^{25}$ +51°, $[\alpha]_{546}^{25}$ +57°, (*c* 0.38, acetone). For ^1H and ^{13}C NMR, see Table III.1.

Photoisomerization of trienal 3 to trienal 1. Trienal 3 was dissolved in 400 μl $CDCl_3$, placed in a 5 mm NMR tube, protected from light, and characterized by 400 MHz NMR as for 3 above. The NMR tube containing trienal 3 in solution was then placed ca. 3 cm from a 254 nm light source (Mineralight UVSL 0.12 Amp) for one hour. Another NMR spectrum of the sample was immediately recorded on the same instrument. By integration of the spectrum, ca. 75% of trienal 3 had isomerized to trienal 1 during the one hour exposure to UV radiation.

Methyl 9-formyl-(15RS)-hydroxy-(6Z,9E,11E,13E)-heptadecatetraenoate (4). From the VC of the crude extract, the fraction which eluted in 40% EtOAc/ cyclohexane (224 mg), devoid of trienals 1 and 3 by TLC analysis, was treated with CH_2N_2 to give a methylated mixture of compounds (305 mg). A second VC yielded a fraction which eluted in 20% EtOAc/cyclohexane (26 mg) which was purified on HPLC (254 nm detection, Waters μPorasil 10 μm, 2 x [300 x 3.9 mm], 4% iPrOH in hexanes) to yield a light oil (14.5 mg, 0.2% yield). FTIR ν_{max}^{film} cm^{-1}: 3500 (br -OH), 2945, 2864, 1734, 1671, 1607, 1440, 1363, 1178, 994, 870, 673. UV λ_{max}^{MeOH} nm: 320 (ε = 37,000). ORD: $[\alpha]_D^{28}$ -1°, $[\alpha]_{578}^{28}$ -1°, $[\alpha]_{546}^{28}$ -2°, $[\alpha]_{436}^{28}$ -3° (*c* 0.46, acetone). For ^1H and ^{13}C NMR data, see Table III.2. Nuclear Overhauser effect (nOe) difference spectroscopy: irradiate δ9.43 (H-9') enhance δ6.85 (H-10) 7%; irradiate δ6.85 (H-10) enhance δ9.43 (H-9') 23%; irradiate δ3.11 (H-8) enhance δ6.66 (H-11, 12) 8%. LR EIMS (probe) 70 eV *m/z* (rel. int.): 320 $[M]^+$ (4), 303 $[M - OH]^+$ (2), 263 (5), 248 (7), 231 (10), 213 (5), 133 (18), 105 (22), 91 (38), 57

(100). HR EIMS obs. $[M]^+$ *m/z* 320.1987 ($C_{19}H_{28}O_4$; -0.1 ppm dev.) GC EIMS of TMSi-ether of **4**, 70 eV *m/z* (rel. int.): 392 $[M]^+$ (20), 367 (7), 335 (37), 245 (9), 237 (11), 179 (15), 131 (20), 91 (23), 73 (100).

Formation of methoxamine derivative of **4**. Methyl ester **4** (1.5 mg) was dissolved in 100 μl of a 10 mg methoxime HCl / ml pyridine solution and left to stand at room temp. for 15 hr. After evaporation of the solvent *in vacuo*, the reaction mixture was dissolved in ether and applied to a TLC plate. Upon development of the plate in 25% EtOAc/hexane, the desired product was visualized with 2',7'-dichlorofluoroscein spray reagent under 254 nm light. The band at 0.3 R_f was scraped from the plate and eluted with Et_2O followed by EtOAc. An aliquot was silylated for mass-spectral structure verification. GC EIMS 70 eV *m/z* (rel. int.): 421 $[M]^+$ (4), 390 $[M - OMe]^+$ (34), 368 (12), 300 $[M - OMe - TMSiOH]^+$ (16), 264 (19), 131 (30), 73 (100).

Steric analysis of methoxamine derivative of **4**. The derivative (1.5 mg) was ozonized for 12 min. in 1 ml $CHCl_3$ at -20° C, then reduced in volume under N_2. The MCC derivative was formed in 50 μl toluene, 50 μl menthoxychlorocarbonate solution, and 10 μl pyridine for 30 min. at room temp. The reaction mixture was partitioned 3X between hexane and H_2O. The hexane solubles were treated with peracetic acid at 50° C overnight, reduced under argon, then dissolved in MeOH and treated with CH_2N_2. The methylated material was purified via pTLC (25% EtOAc/ hexane), eluted with Et_2O, and analyzed by GC and GC EIMS versus standards, revealing at C-15 an enantiomeric composition of almost exactly 50% R and 50% S.

Methyl 9-formyl-15-hydroxy-(9E,11E,13E)-heptadecatrienoate (**5**). A less polar UV-active fraction was collected during HPLC purification of trienal **4**. Further HPLC (RI detection, Versapack 10 μm, 2 x [4.1 x 300 mm], 4% iPrOH in hexanes) yielded an oil (0.4 mg). UV λ_{max}^{MeOH} nm: 318 (ε = 57,000). For ^1H NMR data, see Table III.2.

Methyl (16S)-hydroxy-9-oxo-(10E,12Z,14E)-octadecatrienoate* (**6**). During the HPLC of trienal **5**, a less polar, unstable UV-active oil (0.6 mg) was isolated. UV λ_{max}^{MeOH} nm: 314 (ε = 36,000). LR CI (positive ion., CH_4) GCMS of TMSi-

ether of **6**: 395 $[M + H]^+$ (25), 379 $[M - CH_3]^+$ (11), 351 (10), 323 (22), 305 [M - OTMSi]$^+$ (100), 185 (34), 131 (7), 91 (35), 75 (71), 57 (48). For ^{1}H NMR data, see Table III.3.

Methyl 9-formyl-15-oxo-(6Z,9E,11E,13E)-heptadecatetraenoate (**7**). The original VC fraction which eluted in 33% EtOAc/cyclohexane (212 mg) was treated with CH_2N_2 to yield 270 mg of methylated compounds, which were repurified by a second VC. The fraction which eluted in 15% EtOAc/ cyclohexane (40 mg) contained a UV-active compound which was purified by HPLC (270 nm detection, Waters μPorasil 10 μm, 2 x [3.9 x 300 mm], 4% iPrOH in hexanes). Final cleanup prior to characterization was accomplished via HPLC (RI detection, Versapack 10 μm, 2 x [4.1 x 300 mm], 15% EtOAc in hexanes) to yield an oil (0.73 mg) which at times appeared to crystallize. FTIR ν_{max}^{film} cm^{-1}: 2931, 1736, 1672, 1615, 1436, 1358, 1197, 1170, 1115, 1006, 865. UV λ_{max}^{MeOH} nm: 324 (ε = 53,000). LR EIMS (probe) 70 eV *m/z* (rel. int.): 318 $[M]^+$ (55), 300 (10), 287 $[M - OCH_3]^+$ (18), 261 $[M - C_2H_5C{=}O]^+$ (12), 229 (12), 199 (16), 131 (37), 109 (64), 91 (100), 77 (64). HR EIMS *m/z* obs. $[M]^+$ 318.183 ($C_{19}H_{26}O_4$, -0.3 ppm dev.). For ^{1}H NMR data, see Table III.2.

Methyl (16S)-hydroxy-9-oxo-(10E,12E,14E)-octadecatrienoate (**8**). From the original VC, the fractions eluting from 45-100% EtOAc were combined (745 mg) and treated with CH_2N_2 to give 874 mg of methylated products, which were repurifed via a second VC. The fractions which eluted in 25-30% EtOAc in cyclohexane (80 mg) contained an Et_2O-insoluble gray substance, which was removed by filtration. The Et_2O-soluble portion was injected on HPLC (300 nm detection, Rsil 10 μm, 50 x 1 cm, 40 % EtOAc in hexanes), yielding a UV-active substance (7.2 mg) which charred yellow to purple on TLC upon heating with acidic cupric acetate solution. This fraction was re-injected on HPLC (330 nm detection, Versapack 10 μm, 2 x [4.1 x 300 mm], 3% iPrOH in hexanes, followed by 335 nm detection, Nucleosil 100 5 μm, 4.6 x 250 mm, 15% EtOAc in hexanes) to give a single peak. This pure oil (2 mg) was analyzed using a Bakerbond DNBPG (ionic) 5 μm, 4.6 x 250 mm column, (3% iPrOH in hexanes), from which it eluted as a single peak. FTIR ν_{max}^{film} cm^{-1}: 3340 (br -OH), 2925, 2854, 1742, 1720,

1678, 1597, 1577, 1369, 1218, 1168, 1110, 1029. UV λ_{max}^{MeOH} nm: 312 (ε = 35,000). ORD: $[\alpha]_D^{25}$ +19°, $[\alpha]_{578}^{25}$ +18°, $[\alpha]_{546}^{25}$ +23°, $[\alpha]_{436}^{23}$ +40 (*c* 0.25, acetone). LR EIMS (probe) 70 eV *m/z* (rel. int.) 322 $[M]^+$ (10), 304 $[M - H_2O]^+$ (11), 291 $[M - OCH_3]^+$ (9), 265 (15), 261 (11), 237 (25), 233 (85), 185 (32), 147 (39), 145 (33), 137 (23), 125 (25), 121 (37), 107 (100), 104 (64), 91 (65), 79 (65). HR EIMS obs. $[M]^+$ *m/z* 322.214 ($C_{19}H_{30}O_4$, -1 ppm dev.). For ^1H and ^{13}C NMR data, see Table III.3.

Photo-isomerization of trienone 6 to trienone 8. In the same manner as the isomerization of trienal **3** to trienal **1**, trienone **6** was irradiated with 254 nm UV light for one hour in $CDCl_3$ solution in a 5 mm NMR tube. The NMR spectra recorded before and after irradiation demonstrated that approximately 80 % of trienone **6** had photo-isomerized to trienone **8**.

Hydroxy-epoxy octadecanoids. From the original VC fraction which eluted in 40% EtOAc/ cyclohexane and was subsequently methylated, the subfractions which eluted in 10-15% EtOAc from the second VC of this material were pooled (31 mg) and further purified on HPLC (RI detection, Versapack 10 μm, 2 x [4.1 x 300 mm], 3% iPrOH in hexanes). Additional HPLC purification was attempted (RI detection, Nucleosil 100 5 μm, 4.6 x 250 mm, 1% iPrOH in hexanes + 0.01% HOAc), but the major compound was still accompanied by shouldering peaks. Even after final HPLC purification utilizing the same conditions except for the solvent mixture (0.4% iPrOH in hexanes), there remained a ca. 4:1 mixture (4.3 mg) of two related compounds.

Methyl (12S,13S)-epoxy-(11R)-hydroxy-(9Z,15Z)-octadecadienoate (**9**). ORD: $[\alpha]_D^{25}$ -48°, $[\alpha]_{578}^{25}$ -54°, $[\alpha]_{546}^{25}$ -60°, $[\alpha]_{436}^{25}$ -100°, $[\alpha]_{365}^{25}$ -155° (*c* 0.63, acetone). GCMS analysis of the TMSi-ether of **9** was comparable to that published for the same derivative of the same compound.[279-281] For ^1H and ^{13}C NMR data, see Table III.4.

Methyl (12S,13S)-epoxy-(11R)-(4-bromobenzoyl)-(9Z,15Z)-octadecadienoate (**10**). The above-described mixture (3.5 mg) was dissolved in 3.5 ml dry CH_2Cl_2 and 2 ml triethylamine. Dissolution of 25 mg (11 molar equivalents) of 4-bromobenzoyl chloride and catalytic DMAP into the reaction mixture was

accomplished with stirring. The flask was purged with N_2, sealed, and stirred in the dark for 25 hours. Following removal of solvent in vacuo, the residue from the reaction was triturated in hexanes for application to a VC column. Fractions eluting in 4-8% EtOAc in hexanes (3.5 mg) were injected onto HPLC (245 nm detection, Versapack 10 μm, 2 x [4.4 x 300 mm], 4% EtOAc in hexanes), yielding 1.9 mg (40% yield) of derivative **10**. FTIR ν_{max}^{film} cm^{-1}: 2930, 2856, 1723, 1591, 1461, 1437, 1266, 1173, 1100, 1012, 848, 757. $[\alpha]_D^{24}$ +41° (*c* 0.22, $CHCl_3$), lit. +26° (*c* 0.49, $CHCl_3$).[236] UV λ_{max}^{MeOH} nm: 204, 246 (ε = 25,000; 21,000). CD (EtOH): $\Delta\varepsilon$ = +7.74 (λ_{max} 245 nm), lit. +5.94 (λ_{max} 244 nm).[236] ^{1}H NMR (300 MHz, $CDCl_3$): δ7.91 (2H, *d*, J = 8.5 Hz), 7.57 (2H, *d*, J = 8.5 Hz), 5.71 (1H, *dt*, J = 10.6, 7.5 Hz, H-9), 5.61 (1H, *dd*, J = 9.5, 6.0 Hz, H-11), 5.50 (2H, *m*, H-10, 16), 5.31 (1H, *dt*, J = 10.7, 7.4 Hz, H-15), 3.67 (3H, *s*, Me-ester), 3.06 (1H, *dd*, J = 6.0, 2.1 Hz, H-12), 2.95 (1H, *dt*, J = 5.4, 2.1 Hz), 2.41 (1H, *ddd*, J = 14, 7.4, 5.4 Hz, H-14a), 2.29 (2H, *t*, J = 7.6 Hz, H-2), 2.28 (1H, *m*, H-14b), 2.20 (2H, *m*, H-8), 2.04 (2H, *tt*, J = 7.5, 7.3 Hz, H-17), 1.60 (2H, *m*, H-3), 1.29 (8H, *m*, H-4, 5, 6, 7), 0.96 (3H, *t*, J = 7.5 Hz, H-18). LR EIMS (probe) 70 eV *m/z* (rel. int.): 439 (1), 437 $[M - C_5H_9]^+$ (1), 275 (1), 237 (5), 208 (2), 185 (66), 183 (67), 157 (7), 95 (10), 81 (32), 67 (33), 55 (56).

Methyl (12S,13S)-epoxy-(11R)-(4-bromobenzoyl)-(9Z)-octadecadienoate (**11**). During HPLC purification of derivative **10**, a less polar compound (1.1 mg) was collected and characterized. FTIR ν_{max}^{film} cm^{-1}: 2930, 2857, 1723, 1591, 1464, 1437, 1266, 1198, 1173, 1101, 757. $[\alpha]_D^{24}$ +29° (*c* 0.12, $CHCl_3$). UV λ_{max}^{MeOH} nm: 204, 246 (ε = 20,000; 17,000). CD (MeOH): $\Delta\varepsilon$ = +6.2 (λ_{max} 244 nm). ^{1}H NMR (300 MHz, $CDCl_3$): δ7.91 (2H, *d*, J = 8.6 Hz), 7.57 (2H, *d*, J = 8.6 Hz), 5.71 (1H, *dt*, J = 10.7, 7.5 Hz, H-9), 5.58 (1H, *dd*, J = 9.3, 6.1 Hz, H-11), 5.49 (1H, *dd*, J = 10.7, 9.3 Hz, H-10), 3.67 (3H, *s*, Me-ester), 3.01 (1H, *dd*, J = 6.1, 2.1 Hz, H-12), 2.90 (1H, *dd*, J = 5.6, 2.1 Hz, H-13), 2.29 (2H, *t*, J = 7.5 Hz, H-2), 2.21 (2H, *dt*, J = 7.5, 6.6 Hz, H-8), 1.60 (2H, *m*, H-3), 1.40 (2H, *m*, H-14), 1.30 (14 H, *m*, H-4, 5, 6, 7, 15, 16, 17), 0.89 (3H, *t*, J = 7.0 Hz, H-18). LR EIMS (probe) 70 eV *m/z* (rel. int.): 510 (0.005), 508 $[M]^+$ (0.004), 439 (1), 437 $[M - C_5H_{11}]^+$ (1), 208 (8), 185 (100), 183 (96), 157 (9), 155 (9), 151 (9), 95 (9), 81 (21), 67 (17), 55 (46).

Methyl (9R,10R)-epoxy-(11S)-hydroxy-(12Z)-octadecenoate (**12**). From the original VC fraction which eluted in 33% EtOAc/ cyclohexane and was subsequently methylated, the subfraction which eluted from the second VC in 12% EtOAc/ cyclohexane (17.6 mg) was selected for HPLC (RI detection, Versapack 10 μm, 2 x [4.1 x 300 mm], 15% EtOAc/ hexanes). The major peak from this separation was re-injected on HPLC (RI detection, Nucleosil 100 5 μm, 4.6 x 250 mm, 0.5% iPrOH in hexanes) to yield a colorless oil (2.8 mg). FTIR ν_{max}^{film} cm^{-1}: 3420 (br -OH), 2930, 2857, 1740, 1462, 1438, 1200, 1173, 1026, 903. ORD: $[\alpha]_D^{23}$ +46°, $[\alpha]_{578}^{23}$ +48°, $[\alpha]_{546}^{23}$ +54°, $[\alpha]_{436}^{23}$ +90°, $[\alpha]_{365}^{23}$ +150° (*c* 0.28, acetone). GCMS of the TMSi-ether derivative matched data reported for this compound.[235,279,281] For ^1H and ^{13}C NMR data, see Table III.4.

Methyl (9R,10R)-epoxy-(11S)-(4-bromobenzoyl)-(12Z)-octadecenoate (**13**). Compound **12** (2.25 mg) was derivatized and worked up in the same manner as the bromobenzoylation of compound **9**, except that the reaction proceeded for 51 hours at room temperature. The fraction eluting from preparative VC in 3 % EtOAc in cyclohexane (7 mg) was purified by HPLC using the same conditions as for benzoates **10** and **11**. An additional HPLC run with 3% EtOAc in hexanes yielded a clear, pleasant-smelling oil (0.6 mg, 20% yield). FTIR ν_{max}^{film} cm^{-1}: 2929, 2857, 1723, 1590, 1266, 1173, 1101, 1012, 848, 757. $[\alpha]_D^{18}$ -36° (*c* 0.07, $CHCl_3$). UV λ_{max}^{MeOH} nm: 204, 246 (ε = 24,000; 22,000). CD (MeOH): $\Delta\varepsilon$ = -8.2 (λ_{max} 244 nm). ^1H NMR (300 MHz, $CDCl_3$): δ7.91 (2H, *d*, J = 8.5 Hz), 7.57 (2H, *d*, J = 8.5 Hz), 5.73 (1H, *dt*, J = 10.5, 7.5 Hz, H-13), 5.59 (1H, *dd*, J = 9.3, 6.2 Hz, H-11), 5.49 (1H, *dd*, J = 10.5, 9.3 Hz, H-12), 3.66 (3H, *s*, Me-ester), 3.01 (1H, *dd*, J = 6.2, 2.1 Hz, H-10), 2.90 (1H, *dt*, J = 5.5, 2.1 Hz, H-9), 2.30 (2H, *t*, J = 7.5 Hz, H-2), 2.20 (2H, *bdt*, J = 7.5, 7.5 Hz, H-14), 1.58 (4H, *m*, H-3, 8), 1.41 (2H, *m*), 1.30 (14H, *m*), 0.88 (3H, *t*, J = 6.8 Hz, H-18). LR EIMS (probe) 70 eV *m/z* (rel. int.): 510 (0.016), 508 $[M]^+$ (0.013), 353 (1), 351 (1), 324 (2), 322 (1), 237 (4), 185 (100), 183 (96), 157 (11), 155 (17), 151 (15), 104 (10), 95 (11), 81 (25), 67 (27), 55 (76).

Methyl (9R,10R)-epoxy-(11S)-hydroxy-(12Z,15Z)-octadecadienoate (**14**). A relatively nonpolar, UV-inactive fraction was recovered from the initial HPLC of ketotrienal **7**, and was repurified by HPLC in the same manner as compound **12**

to yield a light yellow oil (2.1 mg). FTIR ν_{max}^{film} cm^{-1}: 3430 (br -OH), 3012, 2932, 2858, 1738, 1461, 1438, 1201, 1174, 1085, 1037, 882, 721. ORD: $[\alpha]_D^{25}$ +43°, $[\alpha]_{578}^{25}$ +47° (*c* 0.18, acetone). LR GC EIMS of TMSi-ether derivative of compound **14**, 70 eV *m/z* (rel. int.): 381 $[M - CH_3]^+$ (0.01), 306 [M - TMSiOH]$^+$ (0.17), 257 (1), 209 (1), 197 (15), 131 (15), 107 (29), 73 (100), 55 (30). For ^1H NMR data, see Table III.4.

Antimicrobial bioassays. A sensitivity-disk bioassay was performed using six micro-organisms form American Type Culture Collection (ATCC): gram positive bacteria *Bacillus subtilis* (ATCC 6081) and *Staphylococcus aureus* (ATCC 12,600); gram negative bacteria *Eschericia coli* (ATCC 11,775), *Pseudomonas aeruginosa* (ATCC 9721), and *Salmonella typhimurium* (ATCC 14,028); and the yeast *Candida albicans* (ATCC 14,053). Sterile paper disks (6 mm diam.) were impregnated with either pure compound or 2 mg crude extract in Et_2O. Et_2O was also applied alone to a negative control disk and allowed to dry. Positive control antibiotic disks (Difco) were used in every bioassay. Disks were placed on inoculated Mueller-Hinton agar plates and incubated at ca. 37° C for 8-16 hr. A clear zone of microbial growth inhibition around a disk indicated positive antibiotic activity. The crude organic extract (Marine Gardens, Oregon, 6 Aug. 1986) was slightly inhibitory towards *Bacillus subtilis* and *Staphylococcus aureus*. Trienal **1** (100 μg/disk) gave a 10 mm diameter zone of inhibition against *Candida albicans*.

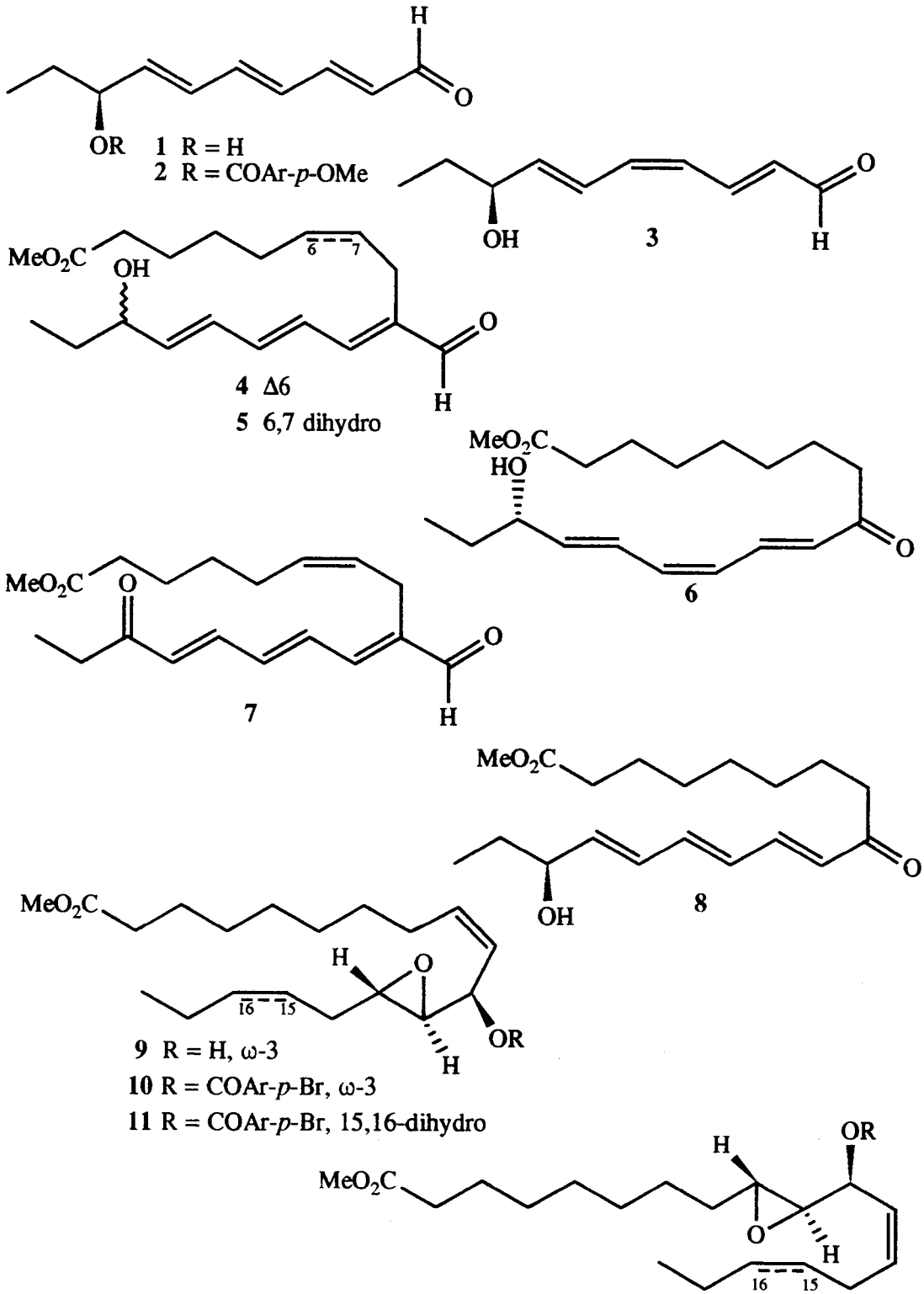


Figure III.1. Chemical structures for Chapter III .

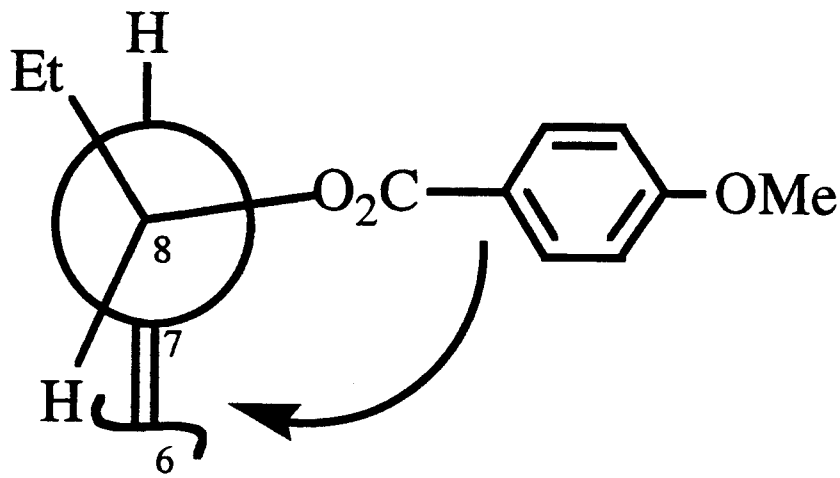


Figure III.2. Newman Projection of Derivative 2.

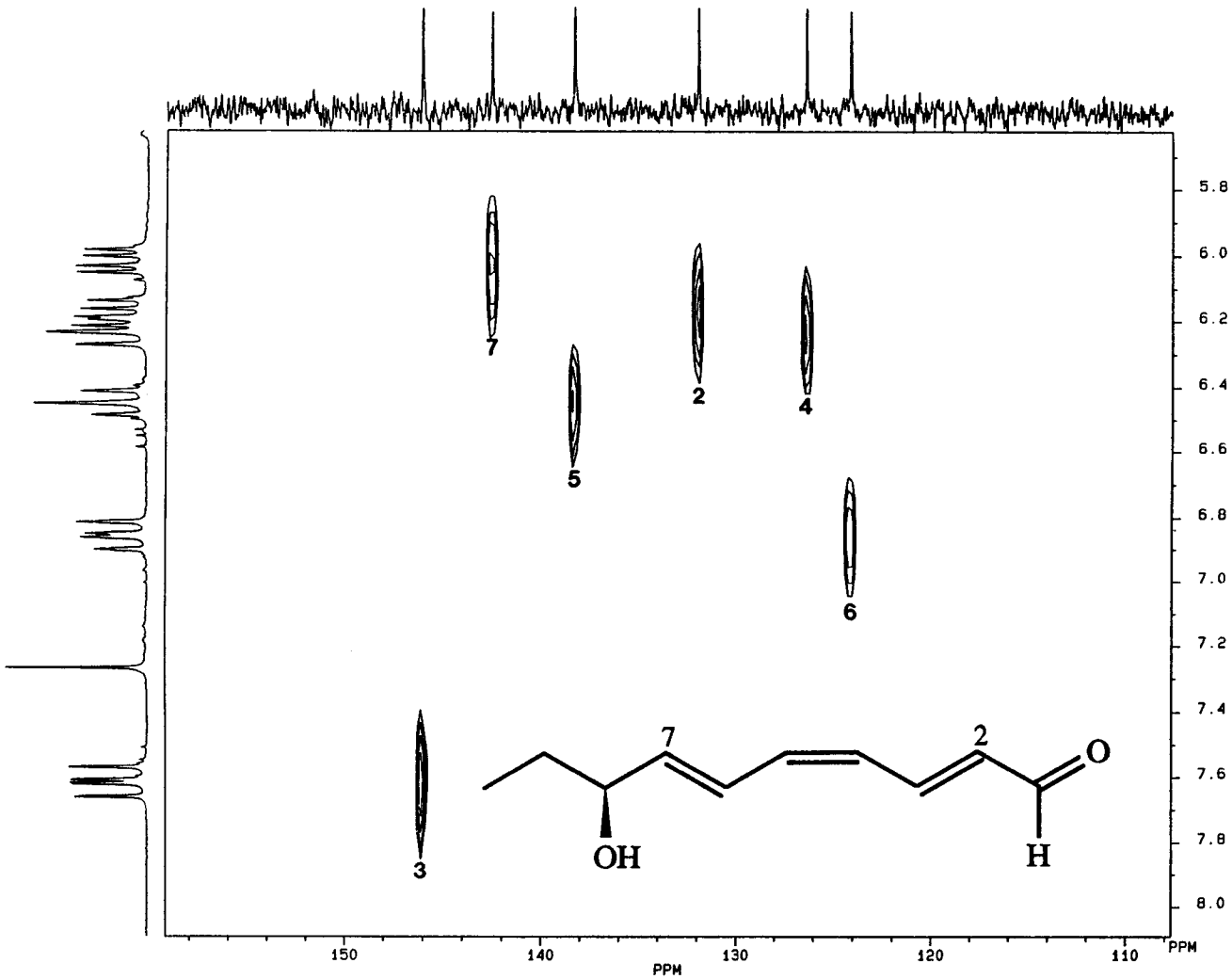


Figure III.3. ^{1}H-^{13}C HETCOR of compound **3** showing expansion of the olefinic region.

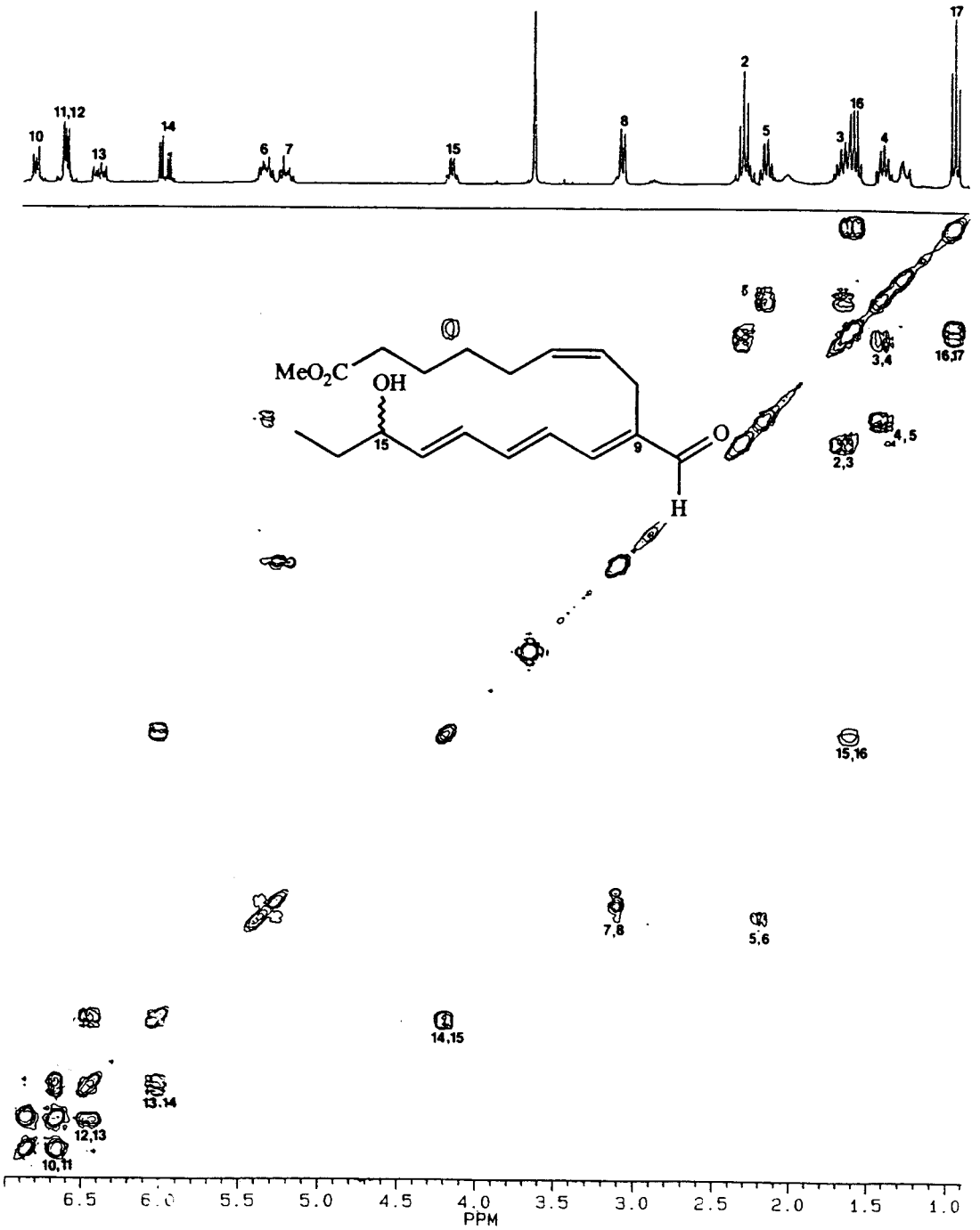


Figure III.4. ^{1}H-^{1}H COSY of branched chain trienal **4**.

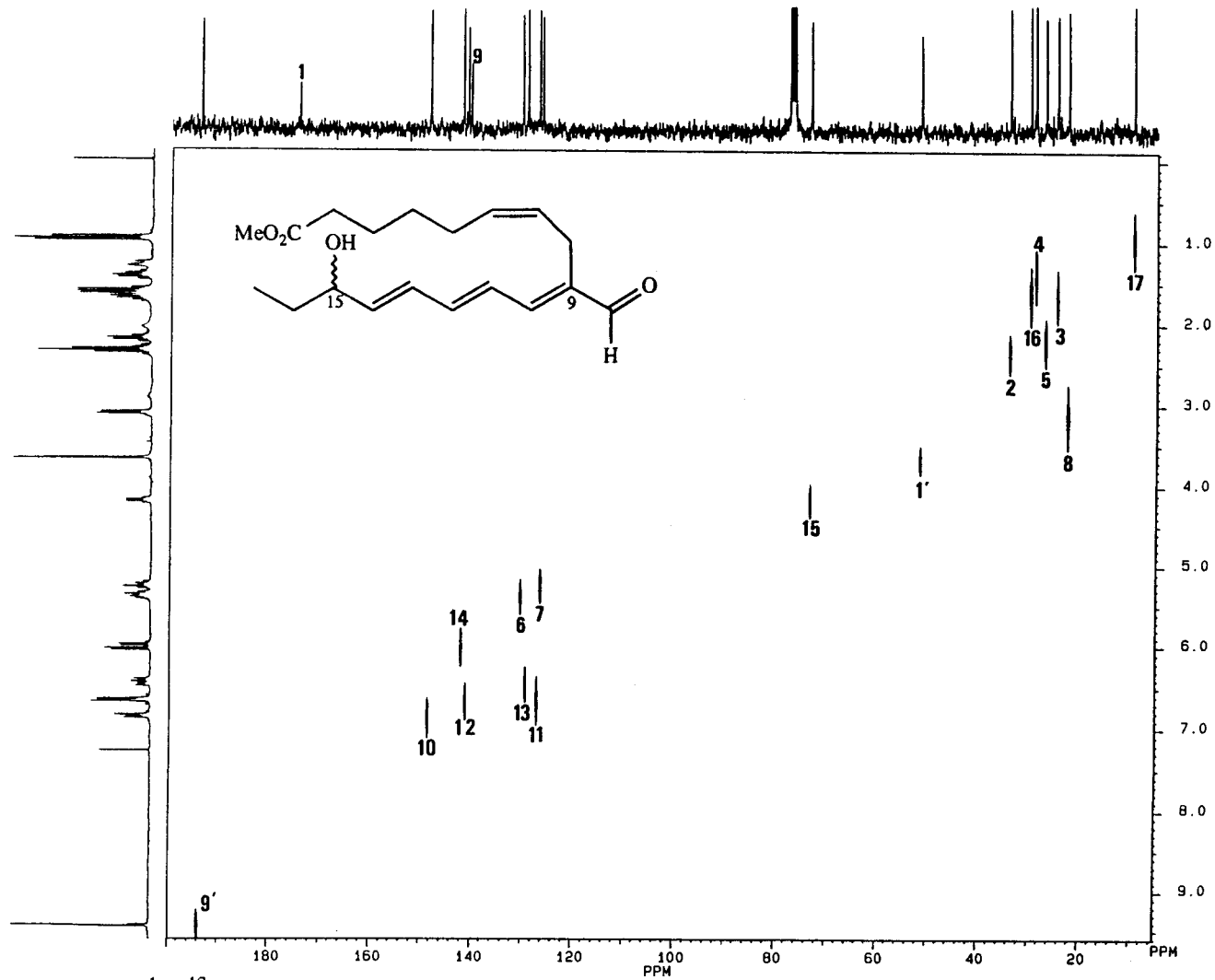


Figure III.5. ^{1}H-^{13}C HETCOR of branched-chain trienal **4**.

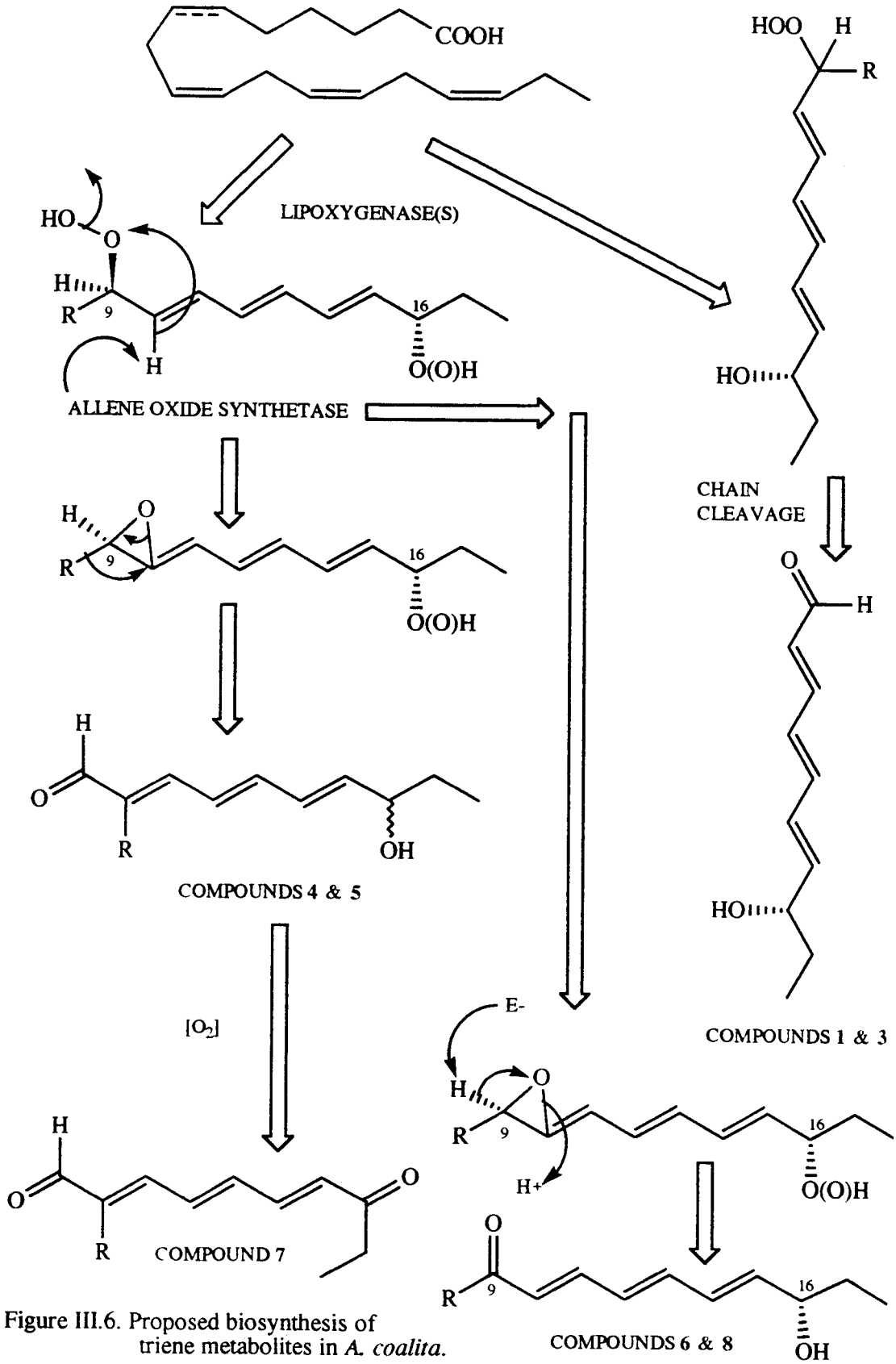


Figure III.6. Proposed biosynthesis of triene metabolites in *A. coalita*.

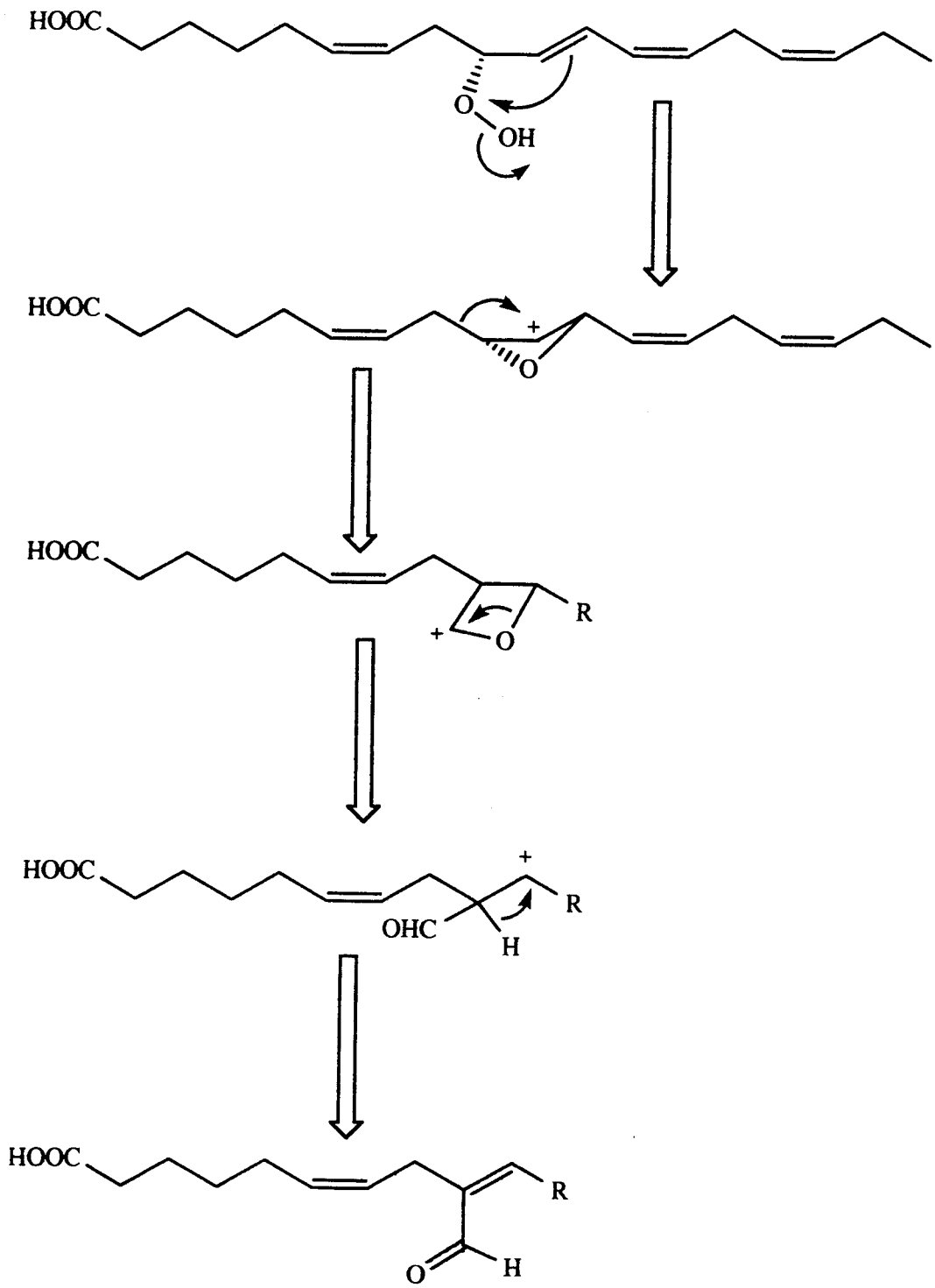


Figure III.6 (Continued)

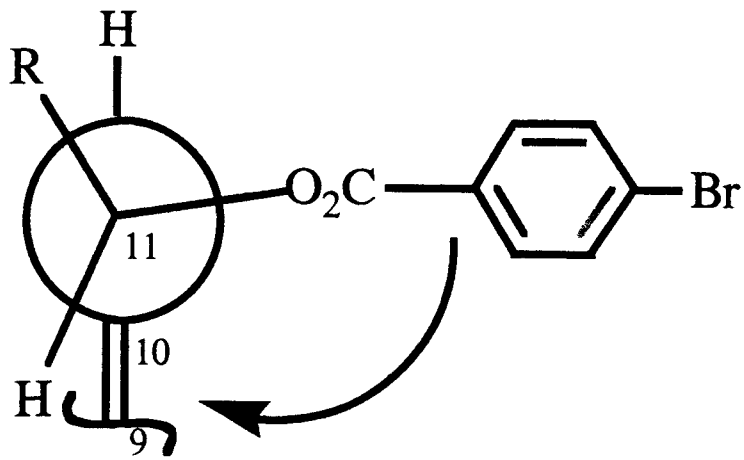


Figure III.7. Newman Projection of Derivatives **10** & **11.**

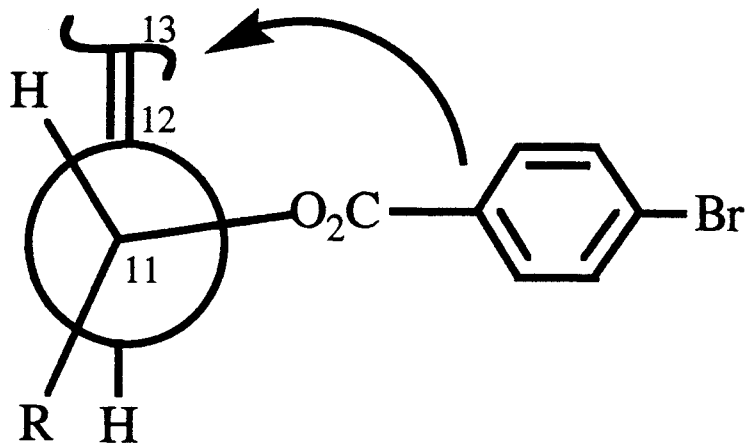


Figure III.8. Newman Projection of Derivative **13.**

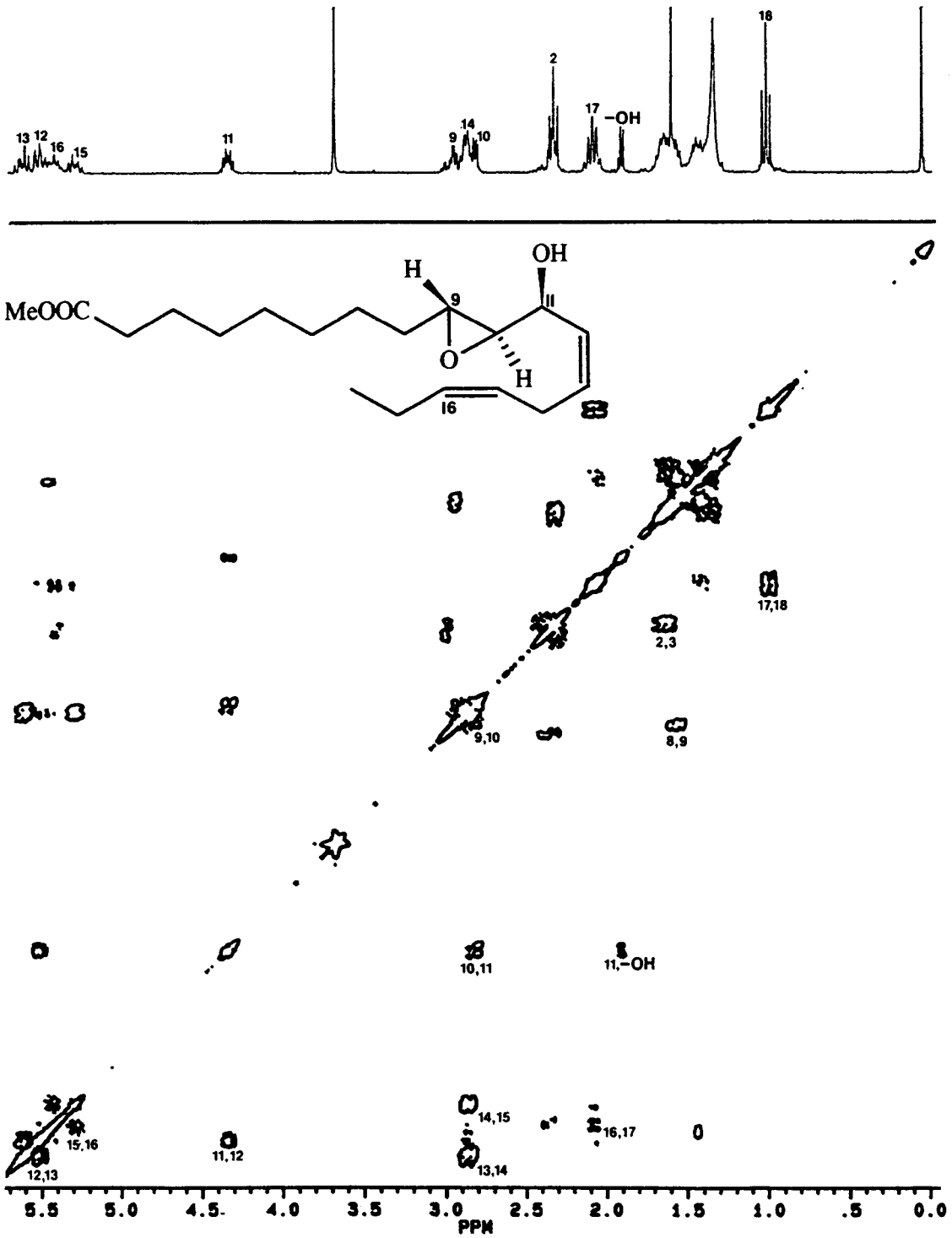


Figure III.9. ^{1}H-^{1}H COSY of compound **14**.

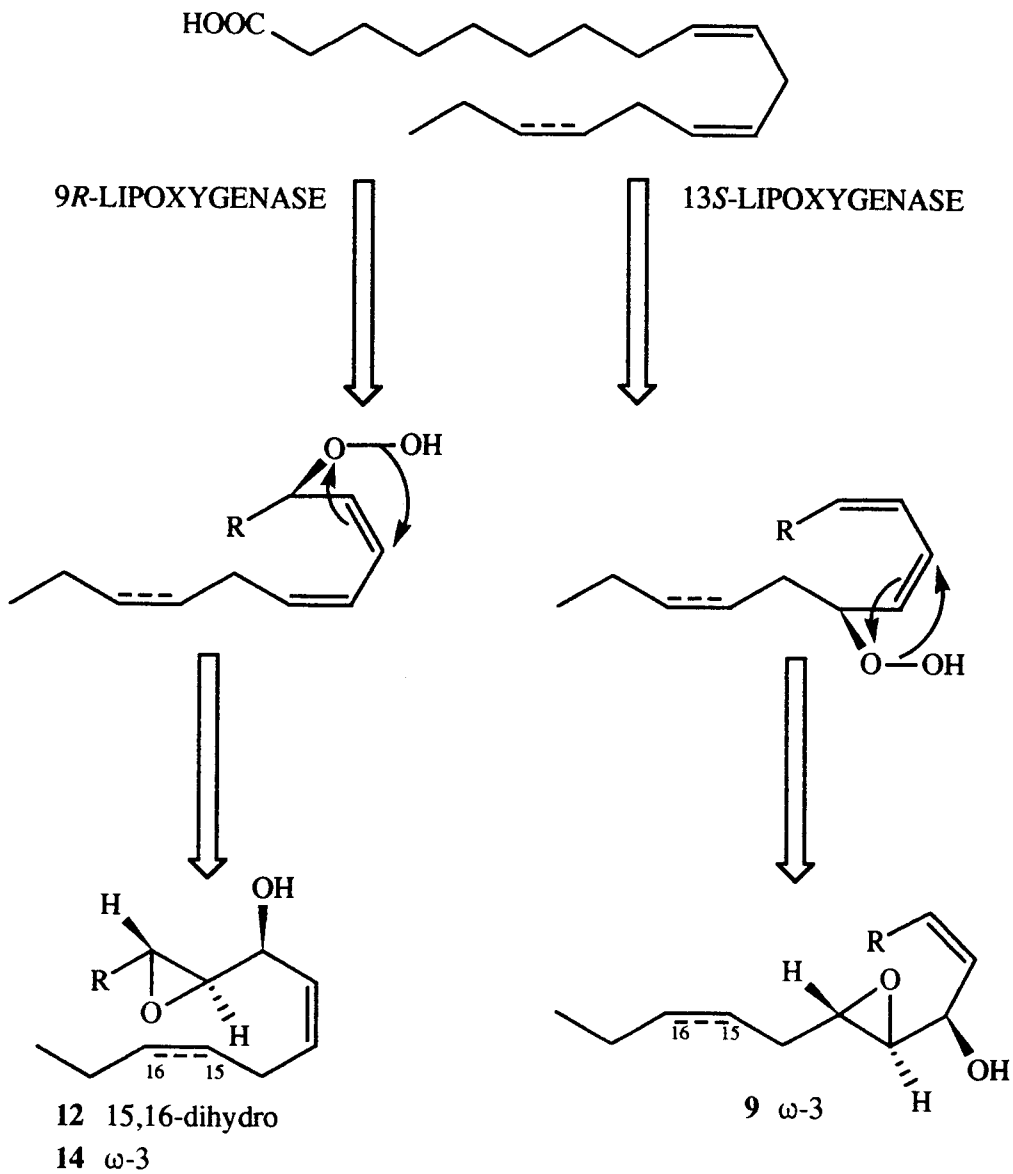
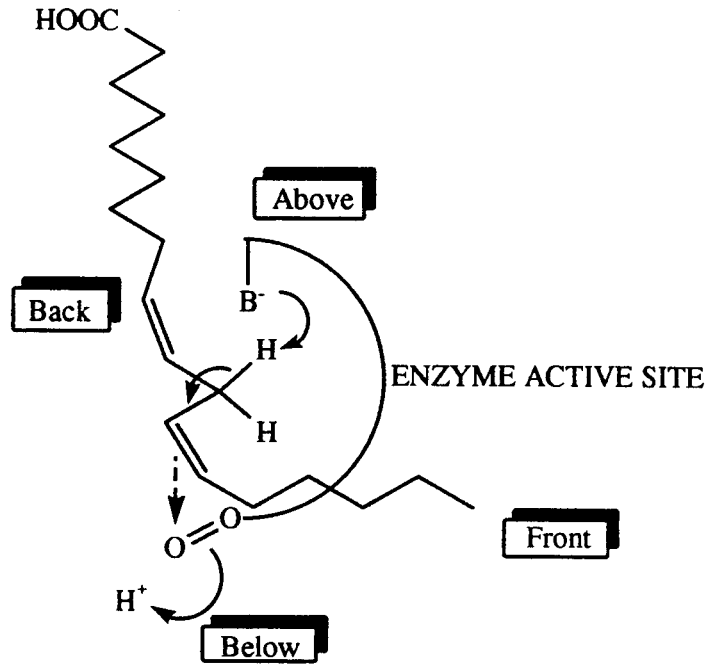


Figure III.10. Proposed formation of hydroxy-epoxy compounds in *A. coalita.*

Pro-*S*-abstraction at C-11 gives 13*S* oxygenation:



Pro-*R*-abstraction at C-11gives 9*R* oxygenation:

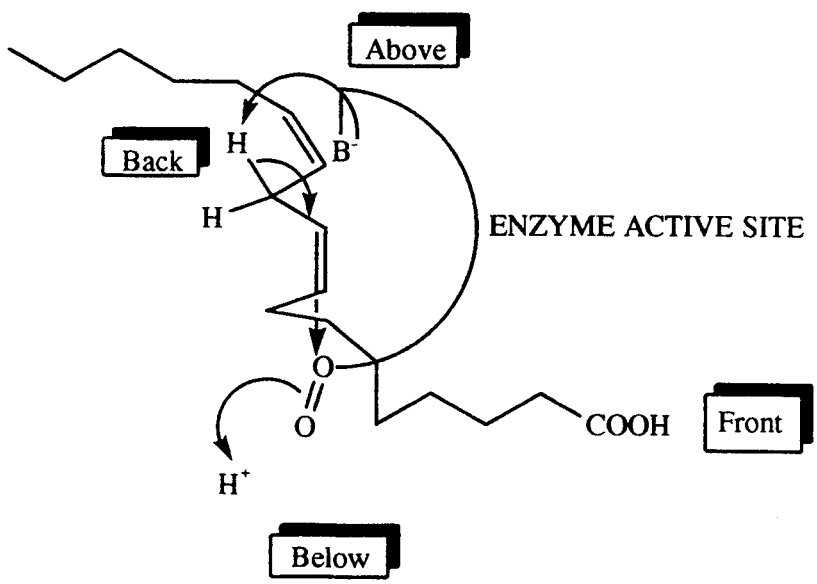


Figure III.11. Alternative formation of hydroxy-epoxy compounds in *A. coalita*.

Table III.1 NMR Data for Two Isomeric Trienal Alcohols from *Acrosiphonia*[a]

	EEE Trienal 1[b]				*EZE* Trienal 3[c]			
C#	δ ^{13}C	δ ^{1}H	m	J (Hz)	δ ^{13}C	δ ^{1}H	m	J (Hz)
1	193.68	9.56	d	7.9	193.74	9.63	d	8.0
2	131.35	6.17	dd	15.3, 7.9	132.00	6.17	dd	15.2, 8.0
3	151.89	7.14	dd	15.3, 11	146.07	7.61	dd	15.2, 11.5
4	129.82	6.44	dd	14.5, 11	126.43	6.23	dd	11.5, 11.5
5	142.01	6.67	dd	14.5, 11	138.34	6.44	dd	11.5, 11.5
6	129.06	6.36	dd	15, 11	124.16	6.85	dd	14.8, 11.5
7	142.59	6.02	dd	15, 6.2	142.55	6.01	dd	14.8, 6.1
8	73.38	4.18	dt	6.7, 6.2	73.42	4.23	dt	6.7, 6.1
9	30.04	1.61	dq	7.3, 6.7	30.14	1.63	dq	7.4, 6.7
10	9.60	0.95	t	7.3	9.61	0.97	t	7.4

[a]All spectra were obtained in $CDCl_3$. Chemical shifts are expressed in ppm relative to TMS internal standard.
[b]Spectra were obtained at 9.398 T. Assignments based on COSY and HETCOR experiments.
[c]Spectra were obtained at 7.047 T. Assignments based on COSY and HETCOR experiments.

Table III.2 NMR Data for Three Novel Octadecanoid Fatty Acids Isolated from *Acrosiphonia coalita* as Methyl Ester Derivatives **4**, **5**, and **7**.[a]

	Branched-Chain Trienal **4**[b]				6,7-dihydro Trienal **5**[c]			Keto-trienal **7**[d]		
C#	δ ^{13}C	δ ^{1}H	m	J (Hz)	δ ^{1}H	m	J (Hz)	δ ^{1}H	m	J (Hz)
1	174.31	----	---	---	----	--	---	----	---	---
2	34.02	2.34	t	7.6	2.30	t	7.3	2.35	t	7.4
3	24.67	1.67	m	---	1.63	tt	7.3, 7.0	1.68	tt	7.6, 7.4
4	29.01	1.43	tt	7.8, 7.4	1.30	m	---	1.44	tt	7.6, 7.6
5	29.98	2.19	dt	7.4, 7.4	1.30	m	---	2.20	dt	7.6, 6.4
6	130.37	5.38	bdt	10.6, 7.4	1.30	m	---	5.42	bdt	10.5, 6.4
7	126.43	5.25	bdd	10.6, 7.2	1.30	m	---	5.23	bdd	10.5, 6.8
8	22.49	3.11	d	7.2	2.34	t	7.2	3.16	d	6.8
9	140.55	----	---	---	----	--	---	----	---	---
10	148.50	6.85	m	---	6.84	m	---	6.89	d	11.6
11	127.05	6.66	m	---	6.65	m	---	7.03	dd	14.5, 11.6
12	141.15	6.66	m	---	6.65	m	---	6.72	dd	14.5, 11.2
13	129.36	6.43	m	---	6.43	m	---	7.27	dd	15.6, 11.2
14	142.10	6.02	dd	14.9, 6.0	5.99	dd	15, 6	6.36	d	15.6
15	73.31	4.19	dt	6.4, 6.0	4.19	dt	7, 6	----	---	---
16	30.02	1.64	dq	7.5, 6.4	1.63	dq	7.5, 7	2.64	q	7.3
17	9.61	0.96	t	7.5	0.96	t	7.5	1.14	t	7.3
9'	193.91	9.43	s	---	9.42	s	---	9.49	s	---
1'	51.53	3.67	s	---	3.66	s	---	3.67	s	---

[a]Chemical shifts ($CDCl_3$) are expressed in ppm relative to TMS internal standard.
[b]Spectra obtained at 7.047 T. Assignments based on COSY and HETCOR experiments.
[c]Spectrum obtained at 7.047 T. Assignments based on comparison to compound **4**.
[d]Spectra obtained at 9.398 T. Assignments based on a COSY experiment and comparison to compound **4**.

Table III.3 NMR Data for Two Novel Octadecanoid Trienones Isolated from *Acrosiphonia coalita* as Methyl Ester Derivatives **6** and **8**.[a]

		EEE Trienone **8**[b]			*EZE* Trienone **6**[c]		
C#	δ ^{13}C	δ ^{1}H	m	J (Hz)	δ ^{1}H	m	J (Hz)
1	174.28	----	--	---	----	--	---
2	34.04	2.30	t	7.5	2.30	t	7.6
3	24.28[d]	1.62	m	---	1.63	m	---
4	29.07[e]	1.32	m	---	1.32	m	---
5	29.07[e]	1.32	m	---	1.32	m	---
6	28.94[e]	1.32	m	---	1.32	m	---
7	24.86[d]	1.62	m	---	1.63	m	---
8	40.72	2.54	t	7.5	2.57	t	7.4
9	200.67	----	--	---	----	--	---
10	129.44	6.17	d	15.4	6.18	d	15.3
11	142.01	7.18	dd	15.4, 10.8	7.64	dd	15.3, 11.3
12	130.54	6.31	dd	14.5, 10.8	6.10	dd	11.3, 11.3
13	140.61	6.60	dd	14.5, 10.6	6.36	dd	11.3, 11.3
14	129.50	6.36	dd	15.3, 10.6	6.84	dd	15, 11.3
15	140.74	5.93	dd	15.3, 6.4	5.92	dd	15, 6
16	73.58	4.16	dt	6.4, 5.1	4.21	dt	6, 5
17	30.08	1.62	m	---	1.63	m	---
18	9.60	0.95	t	7.5	0.96	t	7.5
1'	51.47	3.66	s	---	3.67	s	---

[a]Chemical shifts ($CDCl_3$, 7.047 T) are expressed in ppm relative to TMS internal standard.
[b]Assignments based on COSY and HETCOR experiments.
[c]Assignments based on comparison to trienone **8** and a COSY experiment.
[d,e]Assignments may be interchanged within a given letter.

Table III.4 NMR Data for Three Hydroxy-epoxy Octadecanoid Fatty Acids Isolated from *Acrosiphonia coalita* as Methyl Ester Derivatives **9**, **12**, and **14**.[a]

	Compound 9[b]				Compound 12[c]				Compound 14[d]		
C#	δ ^{13}C	δ ^{1}H	m	J (Hz)	δ ^{13}C	δ ^{1}H	m	J (Hz)	δ ^{1}H	m	J (Hz)
1	174.29	----	---	---	174.27	----	-	---	----	---	---
2	34.03	2.30	t	7.5	34.05	2.31	t	7.5	2.30	t	7.6
3	24.87	1.62	m	---	24.87	1.64	tt	7.5, 7.5	1.63	m	---
4	29.47[e]	1.30	m	---	29.26[f]	1.35	m	---	1.31	m	---
5	29.01[e]	1.30	m	---	29.11[f]	1.30	m	---	1.31	m	---
6	29.01[e]	1.30	m	---	29.11[f]	1.30	m	---	1.31	m	---
7	31.49[e]	1.30	m	---	29.00[f]	1.50	m	---	1.42	m	---
8	27.94	2.08	m	---	31.44	1.54	m	---	1.54	m	---
9	134.42	5.61	bdt	11.1, 7.4	56.94	2.92	dt	5.6, 2.3	2.93	dt	5.6, 2.3
10	127.49	5.50	bdd	11.1, 9	61.41	2.78	dd	5.2, 2.3	2.80	dd	5.1, 2.3
11	67.68	4.29	ddd	9, 5.1, 4.9	67.89	4.29	ddd	9, 5.2, 4.9	4.33	ddd	8.6, 5.1, 4.9
12	60.91	2.83	dd	5.1, 2.2	127.43	5.47	bdd	9, 9	5.50	bdd	10.8, 8.6
13	56.14	2.97	dt	5.4, 2.2	134.55	5.61	bdt	9, 7	5.61	bdt	10.8, 7.4
14	29.18	2.33	bm	---	27.96	2.09	bdt	7, 7	2.85	bdd	7.4, 7.2
15	122.16	5.33	bdt	10.8, 7.4	25.85	1.38	m	---	5.28	bdt	10.7, 7.2
16	134.94	5.52	m	---	31.49	1.30	m	---	5.41	bdt	10.7, 7.2
17	20.66	2.04	m	---	22.50	1.30	m	---	2.06	dq	7.4, 7.2
18	14.17	0.97	t	7.5	14.02	0.89	t	6.7	0.98	t	7.4
1'	51.47	3.67	s	---	51.47	3.67	s	---	3.67	s	---
OH	-----	1.92	d	4.9	----	1.85	d	4.9	1.88	d	4.9

[a]All spectra obtained at 7.047 T in $CDCl_3$, and are expressed in ppm relative to TMS internal standard.
[b]Assignments based on a HETCOR experiment and comparison to literature values.[210,236]
[c]Assignments based on COSY and HETCOR experiments and comparison to a model compound.[119]
[d]Assignments based on a COSY experiment and comparison to compound **12**.
[e,f]Assignments may be interchanged within a particular letter.

CHAPTER IV

LAURENCIONE, A HETEROCYCLE FROM THE OREGON RED ALGA *LAURENCIA SPECTABILIS*

ABSTRACT

Laurencione was isolated from *Laurencia spectabilis* and its structure determined to be an equilibrium mixture of (±)-2-hydroxy-2-methyldihydrofuran-3-one and 5-hydroxy-2,3-pentanedione. On silica gel, laurencione dimerized to optically inactive, crystalline spiro-bis-pinnaketal, a previously reported natural product of *L. pinnatifida*.

INTRODUCTION

Among the marine algae, the genus *Laurencia* produces the greatest diversity of secondary metabolites. For example, tropical *Laurencia* species are best known for their variety of novel halogenated terpenoids and acetogenins,[163] while temperate species have been a source of unusual eicosanoids[195,199] and polyheterocyclic compounds (**1-4**).[170,288-290] Stimulated by our interest in the eicosanoids of the red algae,[52,53] we investigated the only species of *Laurencia* found in Oregon, *L. spectabilis* Post. & Rupr., to determine if it contained eicosanoids similar to those found in the European species, *L. hybrida*.[195,199] While we were able to detect small amounts of 12-HEPE and 12-HETE in the lipid extract of *L. spectabilis*, the major metabolite (16%) of this extract was a new and unrelated molecule which we have named laurencione (**5**). Furthermore, a polar and optically inactive crystalline compound was also isolated and its structure determined by x-ray crystallography to be spiro-bis-pinnaketal (**1**). The x-ray structure of compound **1** had previously been reported from a European collection of *L. pinnatifida*;[288] however, without NMR, IR, or mass spectral data. Consideration of the potential reactivity of laurencione (**5**), and the racemic nature of spiro-bis-pinnaketal (**1**), led to the proposition that **1** might be an artifact of extraction or chromatography. In this chapter, the structure elucidation of two interconverting forms of laurencione (**5** and **6**), the previously un-reported spectroscopic data for spiro-bis-pinnaketal (**1**), and evidence for the artifactual formation of this crystalline compound are presented.

RESULTS AND DISCUSSION

Entire thalli of *L. spectabilis* were collected intertidally at Seal Rock, Oregon, USA, and immediately frozen in dry ice. The 2:1 CH_2Cl_2/MeOH extract was vacuum-chromatographed over silica gel.[200-202] Fractions eluting with 1:1 EtOAc/cyclohexane contained an optically inactive mixture (1.2 g) of compounds **5** and **6**, which could be purified free of contaminants, but not separated from each other by HPLC. High resolution EIMS of this purified mixture gave a single $[M]^+$ at *m/z* 116.0473 for a molecular formula of $C_5H_8O_3$ (-0.3 ppm deviation) while IR showed both hydroxyl and carbonyl stretches. One- and two-dimensional NMR analyses of this material were confounded, however, by the co-existence of two interconverting forms (**5** and **6**) in solution (Table IV.1); for example, ^{13}C NMR spectra displayed five major and five minor resonances.

Acetylation of UV-inactive compounds **5** and **6** (Ac_2O, pyr, RT) gave a single UV- active product (**7**) in high yield which by low resolution EIMS showed a significant ion at *m/z* 158 ($[M - CH_2=C=O]^+$) for a molecular formula of $C_9H_{12}O_5$. The IR spectrum of **7** displayed carbonyl absorbances at $\overline{\nu}$ = 1696 (conjugated), 1746 (acetate) and 1767 cm^{-1} (vinyl acetate), while the UV spectrum of **7** was characteristic of a cisoid enone. The ^1H NMR spectrum of derivative **7** showed resonances attributable to one methyl ketone, two acetate groups, and a trisubstituted olefin adjacent to a deshielded methylene (see Table IV.1). These data in combination with a ^1H-^{13}C correlation experiment and consideration of model compounds[291,292] allowed unequivocal formulation of novel structure **7** and complete data assignment. Formation of acetylation product **7** may be rationalized as the trapped enolic form of the α-diketone 5-hydroxy-2,3-pentanedione (**6**). Indeed, inspection of the ^1H and ^{13}C data for the original natural product mixture identified diketone **6** as the minor component, which by integration of the ^1H-NMR spectrum, obtained in $CDCl_3$, accounted for *ca* 17% of the equilibrium mixture. The remaining major signals in this mixture could then be separately analyzed. A ketal carbon at δ96.49 in the ^{13}C NMR spectrum and

singlet methyl group at δ1.45 in the ^{1}H NMR spectrum defined the remainder (83%) of the laurencione equilibrium mixture as the ketal (±)-2-hydroxy-2-methyldihydrofuran-3-one (**5**). The relationships between these compounds is depicted in Figure IV.2.

More polar fractions (60-100% EtOAc) from the original chromatography deposited pale green crystals (**1**) upon storage at -20°. These were purified by recrystallization to give colorless thick plates, which by low resolution EIMS showed [M]+ at *m/z* 232, to give a molecular formula of $C_{10}H_{16}O_6$, exactly twice that of compound **5** or **6**. However, ^{1}H and ^{13}C NMR analysis of compound **1** showed that it existed as an assortment of interconverting ketals and ketones in solution (major form only in Table IV.2).

Acetylation of crystalline **1**, followed by HPLC, led to the isolation of enone-diacetate **7**, plus a more complex product, **8**, which existed in solution in a single form and was therefore more amenable to NMR study (Table IV.2). However, while an outline of structure **8** could be generated from ^{1}H and ^{13}C NMR data, including a long-range HETCOSY heteronuclear experiment,[293] the existence of three ketals and one ketone precluded its unambiguous structure elucidation. Hence, an x-ray investigation of crystalline **1** was undertaken (Figure IV.3), which defined it to be identical to spiro-bis-pinnaketal, previously isolated from *L. pinnatifida*.[288] With definition of this crystalline material as structure **1**, it became possible to interpret our NMR data for the acetylation product **8** as a ring-opened diacetate derivative of spiro-bis-pinnaketal (**1**).

Two-dimensional TLC analysis led us to suspect that pure laurencione re-arranged on dry silica gel to form a single, stable chemical entity. The potential for laurencione (**5** and **6**) to dimerize into spiro-bis-pinnaketal (**1**), was investigated by streaking a prep. TLC plate with HPLC-purified laurencione and air drying for 30 min prior to development, after which, a major band, more polar than either compound **5** or **6**, was eluted from the silica gel and dissolved in minimal CH_2Cl_2/acetone. Crystals were deposited upon standing and were identified by ^{1}H NMR, IR, and mp as spiro-bis-pinnaketal (**1**). Hence, racemic **1** was produced during silica gel chromatography of laurencione. Although our

isolate of **1** was optically inactive, the original paper reports $[\alpha]_D$ -23° ($CHCl_3$; *c* 0.1).[288]

Remarkably, diketone **6** was invoked, though never isolated, as the biogenetic precursor of a quite unusual tetracyclic polyketal (**2**) from South American *L. chilensis*.[289] Compound **2**, like chilenones A (**3**)[170] and B (**4**)[290] and spiro-bis-pinnaketal **1**, is a racemic crystalline substance composed of linear five-carbon units with oxidation as in laurencione (**5**). These *L. chilensis* compounds (**2-4**) were isolated by silica gel chromatography as extremely minor constituents of sun/air dried and Soxhlet-extracted material, and hence, they too may represent artifacts formed by dimerization or trimerization of a laurencione precursor. Additional evidence for this hypothesis was seen in ^{1}H NMR spectra of polar and UV-active materials from chromatography fractions of the *L. spectabilis* extract, which displayed chilenone-like alkene doublet signals (δ 5.75 and 5.71, J=2 Hz).

The findings presented here could be applied to *Laurencia* taxonomy, and support the placement of *L. pinnatifida* close to the Spectabilis group.[294] Enone-diacetate **7** was toxic to brine shrimp (0.1 mg/ml), and inhibited the growth of *Bacillus subtilis* and *Candida albicans* in the sensitivity disk bioassay (0.1 mg/disk).

EXPERIMENTAL

Melting points (uncorr.) were obtained using a Kofler hot stage. UV absorption spectra were recorded on a Beckman DB-GT spectrophotometer. IR absorption spectra were collected on Nicolet DXB FT 15 and 510 FT-IR spectrophotometers. Optical rotations were calculated using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on Bruker AM 400 and ACP 300 spectrometers, with all chemical shifts reported relative to TMS internal standard. All coupling constants are reported in Hz, and are accurate to within 0.5 Hz. LR EIMS data were obtained on a Finnigan 4023 spectrometer with both direct probe and GC capabilities. HR EIMS data was recorded on a Kratos MS 50 TC. HPLC utilized Waters M6000A and M45 pumps, R401 differential refractometer, and U6K injector. TLC was performed using Merck aluminum-backed TLC sheets with fluorescent indicator (silica gel 60 F_{254}). Chromatography solvents were distilled from glass prior to use. All liquid chromatography utilized the normal phase mode.

Collection, extraction and chromatography. Solvents for chromatography were HPLC-grade or distilled from glass prior to use. Entire algal thalli (227 g dry wt) of *L. spectabilis* were harvested intertidally in March, 1988 at Seal Rock, Oregon, USA, and immediately frozen in dry ice. A voucher specimen has been deposited at the Department of Botany and Plant Pathology Herbarium at Oregon State University. Frozen algae was defrosted by immersion in 2:1 CH_2Cl_2/MeOH. The resulting organic layer was combined with one resulting from gentle heating of the algae in the same solvent mixture, and reduced in vacuo to yield 5.9 g of a crude brown gum. A volatile, acrid, green substance distilled over into the solvent trap during the final stages of rotary evaporation of the crude extract. NMR analysis showed this volatile material to be a complex mixture of compounds. GCMS using mass-spectral library software data identified two of the volatiles as 4-methylpyrimidine and 2-methylpyrazine. The latter compound is a constituent of tobacco[295] and *Annona cherimolia* fruit.[296] An overwhelming aroma is present in fresh specimens of this alga.

Eicosanoid quantification. A 10 mg portion of the crude extract was applied to a silica gel TLC plate and developed in acidified normal phase solvent. UV-active bands were scraped and recovered with Et_2O and EtOAc, then injected on HPLC (237 nm UV detection, μPorasil 10 μm, 2 x [300 x 3.9 mm], 1070:20:16:1 hexane / MeOH / iPrOH / HOAc). By spiking with authentic standards, and constructing a standard curve in the region where the detector response was linear, it was determined that the crude extract contained 0.05% 12-hydroxyeicosapentaenoic and 0.08% 12-hydroxyeicosatetraenoic acids.

Laurencione (**5** & **6**). The remaining crude extract was dry-loaded to a vacuum liquid chromatography column,[200-202] which was eluted with a step-gradient of EtOAc in cyclohexane. The fractions eluting in 1:1 EtOAc/ cyclohexane (1.2 g, 16% yield) were purified to a light green oil by HPLC (RI detection, Rsil 10 μm, 50 x 1 cm, 30% EtOAc in hexanes). FTIR ν_{max}^{film} cm^{-1}: 3400 (-OH), 2994, 1769 (>C=O), 1715, 1406, 1380, 1125, 1086, 1029, 995, 960, 922. GC-EIMS 70 eV *m/z* (rel. int.): 116 $[M]^+$ (3), 99 $[M - OH]^+$ (2), 88 $[M - C{=}O]^+$ (18), 73 $[M - COCH_3]^+$ (28), 61 (29), 43 $[COCH_3]^+$ (100). HR EIMS *m/z* obs. $[M]^+$ 116.0473 ($C_5H_8O_3$, -0.3 ppm dev.) Elemental analysis (Galbraith) found: C, 51.42; H, 6.95. $C_5H_8O_3$ requires C, 51.75; H, 6.95%. For ^1H and ^{13}C NMR data, see Table IV.1.

Enone-diacetate **7**. Acetylation of laurencione (**5** and **6**) yielded an oil as the single product. UV λ_{max}^{MeOH} nm: 227 (ε = 5900). FTIR ν_{max}^{film} cm^{-1}: 3100, 3000, 1767, 1746, 1696, 1433, 1373, 1231, 1202, 1127, 1061, 970. LR EIMS (probe) 70 eV *m/z* (rel. int.): 158 $[M - H_2C{=}C{=}O]^+$ (73), 116 $[M - 2(H_2C{=}C{=}O)]^+$ (29), 115 $[M - H_2C{=}C{=}O - COCH_3]^+$ (12), 103 (16), 98 $[M - H_2C{=}C{=}O - HOAc]^+$ (61), 55 (82), 43 $[COCH_3]^+$ (100). For ^1H and ^{13}C spectra, see Table IV.1.

Spiro-bis-pinnaketal **1**. The polar fractions of the original vacuum chromatography (60-100% EtOAc) deposited pale green crystals upon storage at -20°. Recrystallization from 1:1 Me_2CO/CH_2Cl_2 yielded colorless thick plates, uncorr. mp 124°, lit. 128° [4]. Spiro-bis-pinnaketal **1** crystallized in space group $P\bar{1}$ with a = 7.366(3), b = 7.991(1), c = 10.330(8) Å, α = 108.14(1), β = 94.37(1), γ = 107.70(1)° and one molecule of composition $C_{10}H_{16}O_6$ in the asymmetric unit (see Figure IV.3). A complete Mo data set with $2\theta \leq 45°$ was

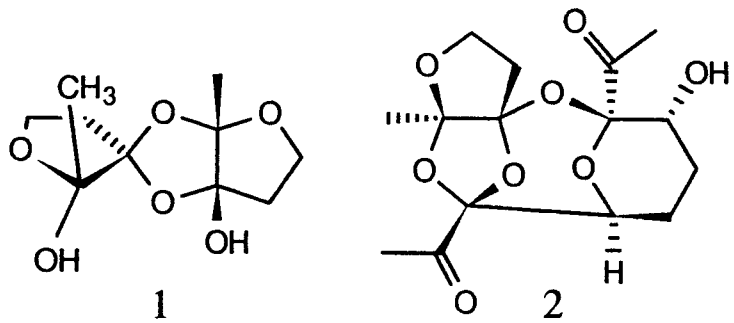
collected, and the 1747 observed reflections (92%, 3.0 σ) were used in refinement. The final R-factor is 4.40%, and archival crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK. FTIR ν_{max}^{film} cm^{-1}: 3400 (-OH), 2988, 1381, 1314, 1234, 1143, 1114, 1096, 1012, 967, 929, 880. LR EIMS (probe) 70 eV, *m/z* (rel. int.): 232 $[M]^+$ (1), 215 $[M - OH]^+$ (100), 197 (14), 189 $[M - COCH_3]^+$ (15), 118 (28), 99 (95), 73 (28). For ^1H and ^{13}C spectra, see Table IV.2.

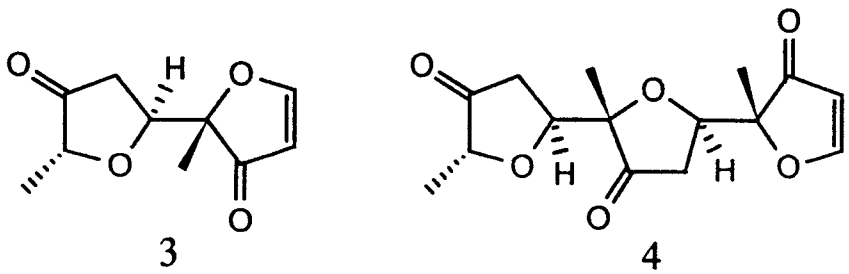
Spiro-bis-pinnaketal acetate derivative **8**. Acetylation of **1** yielded a mixture of compounds which were purified by HPLC (RI detection, Rsil 10 μm, 50 x 1 cm, 40% EtOAc in hexanes). In addition to enone-diacetate **7**, a more polar oily diacetate **8** was recovered from the reaction products. FTIR ν_{max}^{film} cm^{-1}: 2994, 2944, 2900, 1760, 1743, 1735, 1434, 1370, 1311, 1234, 1074, 1012, 897. LR CIMS (CH_4, probe) 70 eV, *m/z* (rel. int.): 317 $[M + H]^+$ (31), 273 $[M - COCH_3]^+$ (7), 257 $[M - OAc]^+$ (38), 213 $[M - COCH_3 - OAc]^+$ (2), 159 (96), 99 (100). HR CIMS (CH_4, probe) 100 eV *m/z*: 317.1218 ($C_{14}H_{21}O_8$, 1.8 mmu dev.), 273.0967 ($C_{12}H_{17}O_7$, 0.7 mmu dev.), 257.1015 ($C_{12}H_{17}O_6$, 1.0 mmu dev.), 213.0755 ($C_{10}H_{13}O_5$, 0.8 mmu dev.). For ^1H and ^{13}C spectra, see Table IV.2.

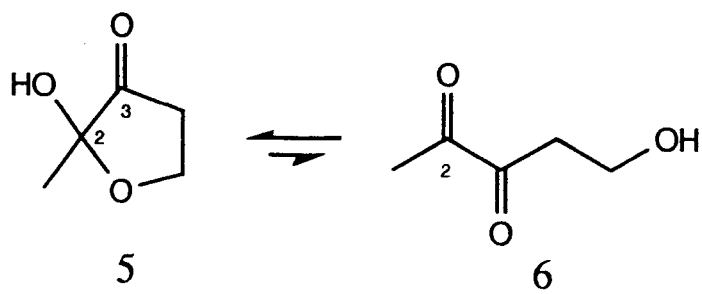
Dimerization of laurencione (**5** & **6**). After purification by HPLC and characterization as above, 30 mg of laurencione was applied to a prep. TLC plate (2 mm) and allowed to dry for 30 min. before development in 100% Et_2O. A band was detected at low R_f (0.16) relative to the starting material by slowly charring a test lane of the developed silica gel plate with 50% H_2SO_4 until a brown color appeared. This test lane was discarded and the band of interest removed. Product was recovered with successive washes of Et_2O, EtOAc, and iPrOH. These eluents were pooled, reduced *in vacuo*, and dissolved in minimal 1:1 Me_2CO/CH_2Cl_2. Crystals were deposited upon standing at -20° and identified as spiro-bis-pinnaketal (**1**) by comparison of mps, ^1H NMR, and FTIR spectra.

Antimicrobial bioassays. A sensitivity-disk bioassay was performed using five micro-organisms form American Type Culture Collection (ATCC): gram positive bacteria *Bacillus subtilis* (ATCC 6081) and *Staphylococcus aureus* (ATCC

12,600); gram negative bacteria *Eschericia coli* (ATCC 11,775) and *Pseudomonas aeruginosa* (ATCC 9721), and the yeast *Candida albicans* (ATCC 14,053). Sterile paper disks (6 mm diam.) were impregnated with either pure compound or 2 mg crude extract using a volatile solvent, such as Et_2O, which was also applied alone to a negative control disk and allowed to dry. Positive control antibiotic disks (Difco) were used in every bioassay. Disks were placed on inoculated Meuller-Hinton agar plates and incubated at ca. 37° C for 8-16 hr. A clear zone of microbial growth inhibition around a disk indicated positive antibiotic activity. The crude organic extract (Seal Rock, Oregon, 26 May 1986) inhibited the growth of all five strains. Enone diacetate **7** (100 μg/disk) gave a 10 mm zone of inhibition against Bs and a 8 mm zone against *Candida albicans*.







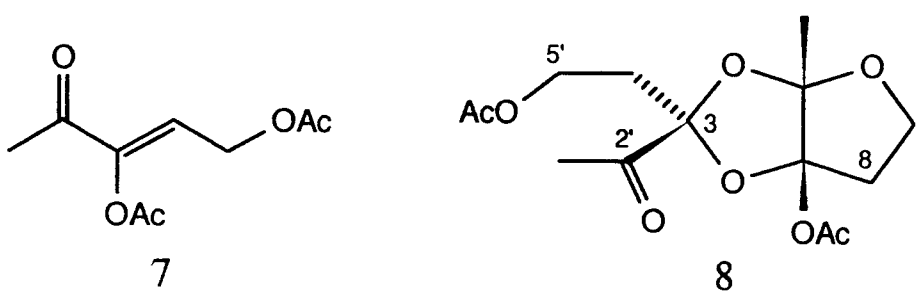


Figure IV.1 Chemical structures for Chapter IV.

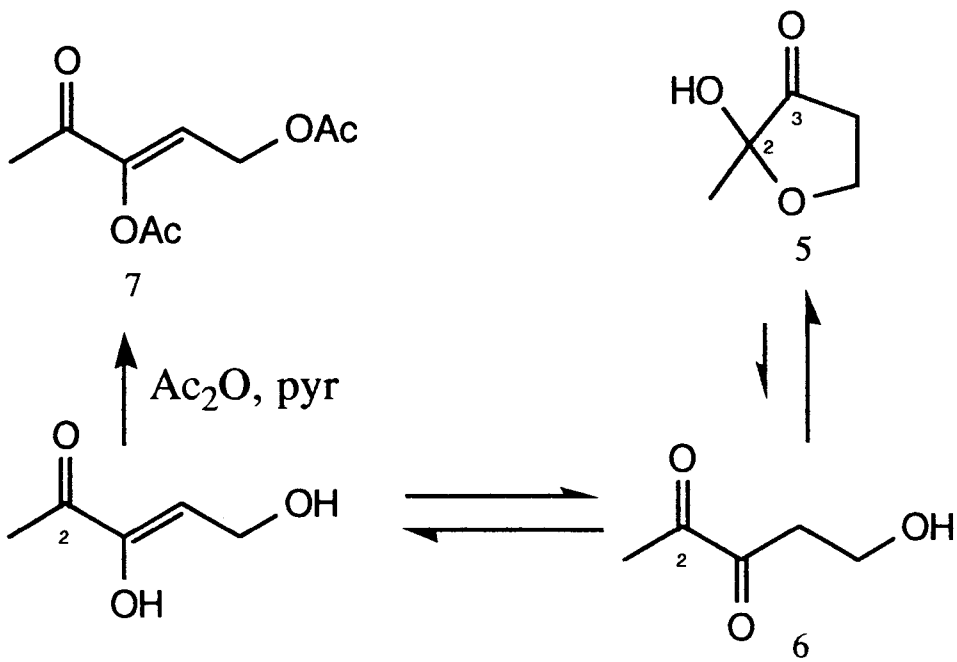


Figure IV.2. Acetylation of laurencione.

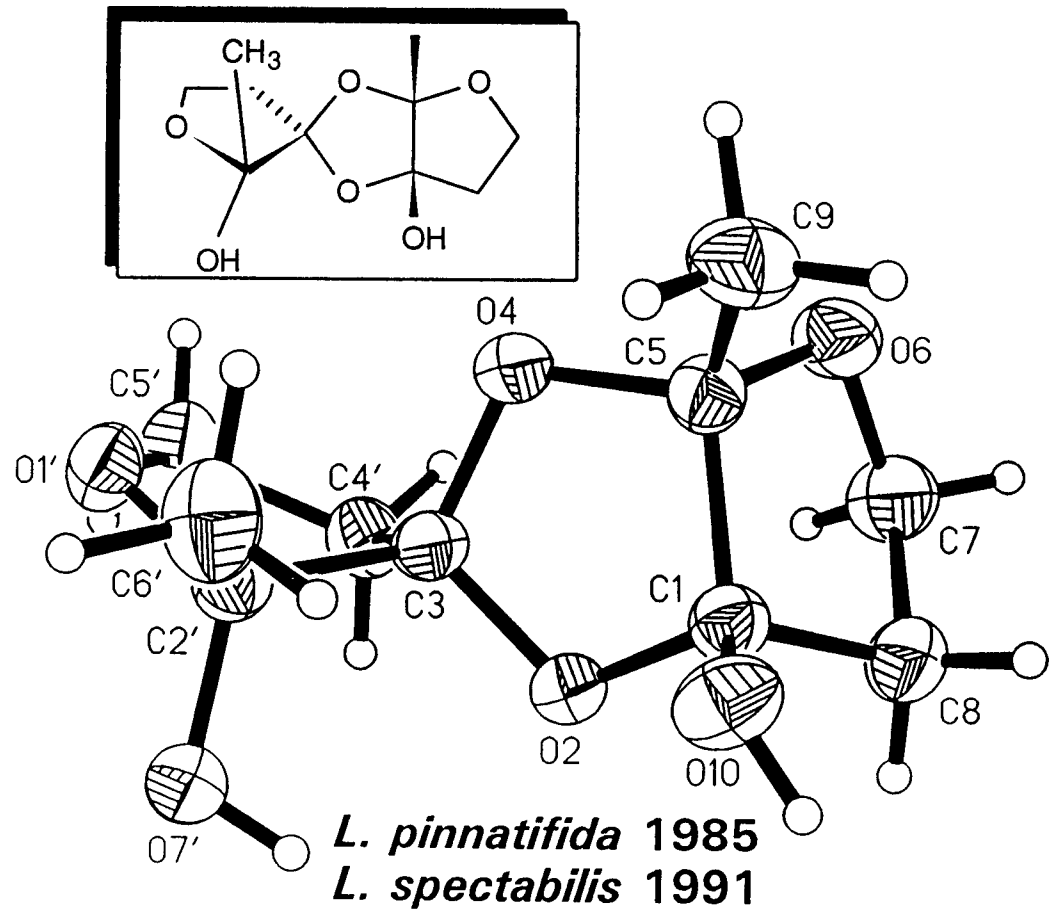


Figure IV.3. ORTEP of Spiro-bis-pinnaketal (**1**).

Table IV.1 NMR Data for Laurencione Ketal **5**, Diketone **6**, and Enone-diacetate **7**:[a]

	Ketal **5**		Diketone **6**		Enone-diacetate **7**	
	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H
1	21.33	1.45 s	23.51	2.34 s	20.13	2.10 s
2	96.49	----	198.44[b]	----	190.82	----
3	209.92	----	197.01[b]	----	146.85	----
4	33.55	2.60 m	38.41	2.99 t (J=5.6)	125.00	6.48 t (J=6.2)
5	61.91	4.24 m	57.31	3.92 t (J=5.6)	58.43	4.75 d (J=6.2)
-OAc	----	----	----	----	170.40	----
					20.57	2.28 s
					168.16	----
					24.82	2.35 s

[a]Chemical shifts ($CDCl_3$) are reported in ppm relative to TMS internal standard on 7.047 T and 9.398 T instruments (coupling constants in Hz). Assignments based on COSY and HETCOR experiments.
b) Assignments may be interchanged.

Table IV.2 NMR Data for Spirobispinnaketal **1** and its Acetate **8**.[a]

	Spirobispinnaketal **1**[b]		Acetate **8**[c]	
C#	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H
1	112.12[d]	----	115.50	----
3	115.20	----	110.78	----
5	110.00[d]	----	111.57	----
7	64.81[e]	3.75 m	66.15	a)3.73 ddd (J=12,8.8,4.7) b)3.51 bt (J=8.8)
8	37.60[f]	2.25 m	36.78	a)2.87 bdd (J=13,4.7) b)1.71 ddd (J=13,12,3.0)
9	21.31[g]	1.31 s	21.65	1.52 s
2'	100.74	----	202.87	----
4'	34.01[f]	1.95 m	34.68	2.04 (second-order m)
5'	61.75[e]	3.75 m	58.89	4.20 (second-order m)
6'	19.80[g]	1.24 s	24.36	1.92 s
-OAc	-----	----	167.83	----
			20.67	1.59 s
			169.74	----
			20.22	1.61 s

a) All chemical shifts are reported in ppm relative to TMS standard on a 7.047 T instrument (coupling constants in Hz). Carbon numbering follows the original report of spirobispinnaketal.[288]
b) DMSO-d_6.
c) Benzene-d_6. Assignments by COSY, HETCOR, LR HETCOSY[293], and COLOC experiments.
d-g) Assignments may be interchanged within the same letter.

CHAPTER V

3-(HYDROXYACETYL)INDOLE, A PLANT GROWTH REGULATOR FROM THE OREGON RED ALGA *PRIONITIS LANCEOLATA*

ABSTRACT

A plant growth regulatory substance, 3-(hydroxyacetyl)indole, and two related tryptophan metabolites have been isolated from the red marine alga *Prionitis lanceolata*. Their structures were determined by spectrochemical methods and comparisons to published data. We find that 3-(hydroxyacetyl)indole stimulates lettuce seedling root growth, and report its first isolation from either a plant or marine source.

INTRODUCTION

For centuries, marine algae have been applied to croplands as a method for improving yields and plant vigor. Some of these agricultural benefits have been attributed to the auxin content of the seaweeds.[297] From the marine environment, a variety of tryptophan metabolites have been reported from bacteria, animals, and plants, including red algae.[173] However, the biological roles of these indole natural products in the algae are less clearly defined than in the higher plants. In the brown alga *Undaria pinnatifida* Suringar, an auxin content approximating that of higher plants was reported.[298] More recently, caulerpin, a putative dimer of indole-3-acrylic acid found in several species of the green alga *Caulerpa*, was demonstrated to promote lettuce-seedling root elongation.[299] In the same assay, the known compound 3-(hydroxyacetyl)indole (**2**), isolated from the red alga *Prionitis lanceolata* (Harv.) Harvey, showed significant activity.

RESULTS AND DISCUSSION

During a survey of Oregon seaweeds for potential biomedicinals, we found that crude organic extracts of *P. lanceolata, P. linearis* Kyl., and *P. lyalli* Harv. demonstrated antimicrobial activity, brine-shrimp toxicity, and unusual TLC characteristics. Therefore, we collected *P. lanceolata* en masse from Cape Perpetua on the Oregon coast, where it grows profusely on rocks and in tidepools during the summer months. Vacuum chromatography of the $CHCl_3$/MeOH extract yielded a series of fractions, some of which contained UV-active compounds that charred a bright, permanent pink color on TLC after spraying with 50% H_2SO_4. The most abundant pink-charring compound recrystallized from acetone and was identified as indole-3-carboxaldehyde (**1**), a known degradation product of indole-3-acetic acid (IAA) in higher plants[300] and fungi.[301] The structure of compound **1** was determined by comparison with published ^{1}H-NMR[302] and mass-spectra,[303] as well as by TLC versus an authentic standard (Aldrich).

A more polar metabolite, compound **2**, was purified by HPLC and shown to contain an indole nucleus by UV and ^{1}H-NMR analyses. The ^{13}C-NMR spectrum displayed a carbonyl (δ194.49), which by IR (ν = 1645 cm^{-1}) was in conjugation with the indole ring system and hydrogen-bonded. Furthermore, the side chain contained a methylene (δ4.70) which was coupled by 5 Hz to a hydrogen-bonded hydroxyl proton (δ3.91). The mass spectrum of the semi-synthetic bis-TMSi derivative of **2** matched that published for the same derivative of 3-(hydroxyacetyl)indole, which had been previously identified as a product of tryptophan side-chain oxidase and alcohol dehydrogenase pathways in the bacterium *Pseudomonas fluorescens*.[304]

Compound **2** has recently been produced as an intermediate in the synthesis of the topsentins, a series of antiviral and antitumor bisindoles from Caribbean deep-sea sponges.[305] Research in the Soviet Union has shown synthetic 3-(hydroxyacetyl)indole (**2**) to be a potent stimulant of the human central nervous system with effects similar to those of amphetamines.[306]

A third indole metabolite, compound 3, was isolated as the semi-synthetic methyl ester (4). Analysis of the ^{1}H-NMR, UV, and mass spectra[298,303] of derivative 4 identified the natural product 3 as indole-3-carboxylic acid, a degradation product of IAA which has previously been detected in brown[298] and red algae.[307]

Because of the structural similarity of metabolite 2 to IAA, as well as to marine sponge-derived compounds found to act as plant growth regulators,[308] we sought to test compound 2 in the lettuce-seedling root elongation assay.[299] At a concentration of 10^{-10} M, natural product 2 promoted significant root elongation versus the control treatment, but was not as potent as IAA. By two different assays, (*Avena* coleoptile growth, competition with IAA uptake into sealed zucchini plasma membrane vesicles), compound 2 showed no true auxin activity. However, because the concentration of metabolite 2 in the alga (approx. 20 ppm on a dry wt basis) is greater than would be necessary for normal terrestrial or marine plant growth regulation, we speculate that 3-(hydroxyacetyl)indole (2) may play a role in the chemical ecology of this alga. By TLC analysis, *P. lyalli* and *P. linearis*, which also grow in abundance on the Oregon coast, exhibit a similar over-production of indole metabolites. Along the Oregon coastline, these *Prionitis* species are remarkably free of any visible epiphytic growth.[309] In the case of *P. lanceolata*, the active principle(s) in the antimicrobial and brine-shrimp assays have not yet been identified.

Since this research described in this chapter was published,[186] indole-3-carboxaldehyde (1), a constituent of cabbage leaves, was shown to be 100% effective at inhibiting the germination of watercress seeds at the concentration of 80 μg/ml.[310] Thus aldehyde 1 may inhibit settlement and overgrowth of *Prionitis* by other marine plants.

EXPERIMENTAL

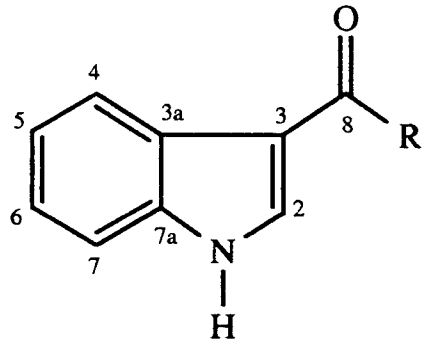
Melting points (uncorr.) were obtained using a Kofler hot stage. UV spectra were recorded on a Beckman DB/GT and a Milton Roy Spectronic 3000 Array spectrophotometer. IR spectra were recorded using a Nicolet 510 FTIR spectrophotometer. Low resolution mass spectra were obtained through the service provided by the Environmental Health Sciences Department at Oregon State University on Kratos MS 50 TC and Finnigan 4023 spectrometers. NMR spectra were obtained using Bruker AM 400 and ACP 300 spectrometers. All NMR shifts are reported relative to TMS internal standard. Coupling constants are expressed in Hertz (Hz) and are accurate to within 0.5 Hz. All chromatography solvents were distilled from glass prior to use.

Collection, extraction, and chromatography. Entire algal thalli were harvested in the intertidal surf zone of Cape Perpetua, Oregon in August 1986, and immediately frozen with dry ice. A voucher specimen has been deposited at the Department of Botany and Plant Pathology Herbarium at Oregon State University. Freshly defrosted algae (585 g dry wt) was macerated and extracted 3X in 2:1 $CHCl_3$/MeOH by gently boiling for 15 min. Filtration and removal of solvent in vacuo yielded 7.8 g of crude organic extract which was then subjected to vacuum chromatography over silica gel using EtOAc in TMP. By TLC, the resulting polar fractions contained mixtures of UV-active metabolites. These fractions were therefore recombined for vacuum chromatography over silica gel using MeOH/CH_2Cl_2. The fractions which eluted in 100% CH_2Cl_2 contained the UV-quenching indole-3-carboxaldehyde (**1**) which on TLC charred to a permanent, vivid pink color with 50% H_2SO_4. Metabolite **1** (20 mg) was recrystallized from Me_2CO, mp 198-203° (lit. 195-197° [Aldrich]). ^{1}H NMR (400 MHz, DMSO-d_6): δ12.16 (1H, *br s*, H-1), 9.94 (1H, *s*, H-8), 8.29 (1H, *d*, J = 2.8 Hz, H-2), 8.11 (1H, *d*, J = 7.6 Hz, H-4), 7.52 (1H, *d*, J = 7.6 Hz, H-7), 7.24 (2H, *m*, H-5 and H6).

3-(hydroxyacetyl)indole (**2**). A more polar metabolite, which eluted in up to 1% MeOH in CH_2Cl_2, was further purified by HPLC (UV detection, Alltech 10

μm, 1 x 50 cm, 75% EtOAc in iso-octane). Removal of solvent yielded 5.7 mg of crystalline needles, mp 173-174° (EtOAc), lit.[305] 173-174°, which were decolorized by trituration in CCl_4 and decantation of the solvent. IR ν_{max}^{film} cm^{-1}: 3225, 2921, 2851, 1645, 1526, 1428, 1246, 1074, 926, 750; UV λ_{max}^{MeOH} nm: 212, 240, 294 (ε = 10,200; 5500; 4500; ^{1}H NMR (400 MHz, acetone-d_6): δ11.17 (1H, *br s*, H-1), 8.33 (1H, *d*, J=3.3 Hz, H-2), 8.30 (1H, *m*, H-4), 7.55 (1H, *m*, H-7), 7.26 (2H, *m*, H-5, 6), 4.73 (2H, *d*, J = 5.0 Hz, H-9), 3.91 (1H, *t*, J = 5.0 Hz, -OH); ^{13}C NMR (100.61 MHz, acetone-d_6): δ194.49 (qC, C-8), 137.65 (qC, C-7a), 133.44 (CH, C-2), 126.58 (qC, C-3a), 124.07 (CH, C-4, 5, or 6), 122.96 (CH, C-4, 5, or 6), 122.44 (CH, C-4, 5, or 6), 114.37 (qC, C-3), 112.90 (CH, C-7), 65.85 (CH_2, C-9). Carbon multiplicities were determined by a DEPT experiment.

Indole-3-carboxylic acid (**3**). The most polar major metabolite, which eluted in 1-2% MeOH in CH_2Cl_2, also acid-charred bright pink on TLC. After prep-TLC (2 mm, UV detection), impure **3** was treated with ethereal CH_2N_2 to yield the methyl ester (**4**), which was purified by HPLC (Alltech Versapack 10 μm, 2 x [4.1 mm x 30 cm], 35% EtOAc in hexanes) to yield 3 mg of crystalline plates, mp 150° (Me_2CO). UV λ_{max}^{MeOH} nm: 229, 279, 285 sh (ε = 9800, 9800, 9100); ^{1}H NMR (300 MHz, $CDCl_3$): δ8.61 (1H, *br s*, H-1), 8.20 (1H, *m*, H-4), 7.93 (1H, *d*, J = 3.0 Hz, H-2), 7.42 (1H, *m*, H-7), 7.28 (2H, *m*, H-5, 6), 3.93 (3H, *s*, H-9).



1 R = H

2 R = CH_2OH

3 R = OH

4 R = OCH_3

Figure V.1. Chemical structures for Chapter V.

BIBLIOGRAPHY

1. Scheuer, P.J. *Sea Grant Quarterly*, Univ. of Hawaii Sea Grant College Program **1986**, *8*, 1-8.

2. Hoffmann, R. *Am. Scientist* **1990**, *78*, 308-309.

3. Fenical, W. *Science* **1982**, *215*, 923-928.

4. Haslam, E. *Nat. Prod. Rep.* **1986**, *3*, 217-249.

5. Herbert, R.B. *The Biosynthesis of Secondary Metabolites*, 2nd ed.; Chapman and Hall: London, **1989**; 231 pp.

6. Mann, J. *Secondary Metabolism*, 2nd ed.; Clarendon: Oxford, **1987**, 374 pp.

7. Luckner, M. *Secondary Metabolism in Microorganisms, Plants, and Animals*, 2nd ed., Springer-Verlag: Berlin, **1984**; 576 pp.

8. Scheuer, P.J. *Chemistry of Marine Natural Products*; Academic: New York, **1973**; 201 pp.

9. Scheuer, P.J., Ed. *Marine Natural Products: Chemical and Biological Perspectives*; Academic: New York, **1978-1983**; Vols. I-V.

10. Scheuer, P.J., Ed. *Bioorganic Marine Chemistry*; Springer-Verlag: Berlin, **1987-1991**; Vols. 1-4.

11. Faulkner, D.J. *Nat. Prod. Rep.* **1984**, *1*, 251-280.

12. Faulkner, D.J. *Nat. Prod. Rep.* **1984**, *1*, 551-598.

13. Faulkner, D.J. *Nat. Prod. Rep.* **1986**, *3*, 1-33.

14. Faulkner, D.J. *Nat. Prod. Rep.* **1987**, *4*, 539-576.

15. Faulkner, D.J. *Nat. Prod. Rep.* **1988**, *5*, 613-663.

16. Faulkner, D.J. *Nat. Prod. Rep.* **1990**, *7*, 269-309.

17. Faulkner, D.J. *Nat. Prod. Rep.* **1991**, *8*, 97-147.

18. Scheuer, P.J. *Med. Res. Rev.* **1989**, *9*, 535-545.

19. Cardellina, J.H., II *Pure Appl. Chem.* **1986**, *58*, 365-374.

20. Rinehart, K.L. in *Biomedical Importance of Marine Organisms*, Fautin, D.G., Ed.; California Academy of Sciences Memoir No. 13: San Francisco, **1988**; pp. 13-22.

21. Begley, S.; Joseph, N. *Newsweek* **1990**, May 21, pp. 77-78.

22. Gurr, M.I.; Harwood, J.L. *Lipid Biochemistry: An Introduction*, 4th ed.; Chapman and Hall: London, **1991**; 406 pp.

23. Stryer, L. *Biochemistry*, 3rd ed.; W.H. Freeman: New York, **1988**, Chapters 20 and 23.

24. Vance, D.E.; Vance, J.E., Eds. *Biochemistry of Lipids and Membranes*; Benjamin / Cummings: Menlo Park, **1985**; 593 pp.

25. Hitchcock, C.; Nichols, B.W. *Plant Lipid Biochemistry: The Biochemistry of Fatty Acids and Acyl Lipids with Particular Reference to Higher Plants and Algae*; Academic: London, **1971**; 387 pp.

26. Montgomery, R.; Conway, T.W.; Spector, A.A. *Biochemistry: A Case-Oriented Approach*, 5th ed.; C.V. Mosby: St. Louis: **1990**; Chapters 10-12.

27. Stumpf, P.K., Ed. *The Biochemistry of Plants: A Comprehensive Treatise*; Academic: New York, **1987**; Vol. 9, 363 pp.

28. Gunstone, F.D.; Harwood, J.L.; Padley, F.B. *The Lipid Handbook*; Chapman and Hall: London, **1986**; 885 pp.

29. Lands, W.E.M. *Fish and Human Health*; Academic: Orlando, **1986**; 170 pp.

30. Kinsella, J.E. *Seafoods and Fish Oils in Human Health and Disease*; Marcel Dekker: New York, **1987**; 317 pp.

31. Kettler, A.H.; Baughn, R.E; Orengo, I.F.; Black, H.; Wolf, J.E. *J. Am. Acad. Dermatol.* **1988**, *18*, 1267-1273.

32. Kremer, J.M. *J. Musculoskel. Med.* **1989**, *6*, 13-22.

33. Kromhout, D.; Bosschieter, E.B.; Coulander, C. *N. Engl. J. Med.* **1985**, *312*, 1205-1209.

34. Rudin, D.O.; Felix, C. *The Omega-3 Phenomenon: The Nutrition Breakthrough of the '80s*; Rawson: New York, **1987**; pp. 31, 76-80.

35. Gunstone, F.D. *Nat. Prod. Rep.* **1987**, *4*, 95-112.

36. Cohen, Z.; Vonshak, A.; Richmond, A. *J. Phycol.* **1988**, *24*, 328-332.

37. Serhan, C.N.; Wong, P.Y.; Samuelsson, B. *Prostaglandins* **1987**, *34*, 201-204.

38. Smith, D.L.; Willis, A.L. *Lipids* **1987**, *22*, 983-986.

39. Smith, W.L.; Borgeat, P.; Hamberg, M.; Roberts, L.J.; Willis, A.L.; Yamamoto, S.; Ramwell, P.W.; Rokach, J.; Samuelsson, B.; Corey, E.J.; Pace-Asciak, C.R. *Methods Enzymol.* **1990**, *187*, 1-12.

40. Gerwick, W.H.; Moghaddam, M.F.; Hamberg, M. *Arch. Biochem. Biophys.* **1991**, *290*, in press.

41. Willis, A.L. in *CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids*, Willis, A.L., Ed.; CRC: Boca Raton, **1987**; Vol. I, p.4.

42. Schwartzman, M.L. in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Samuelsson, B.; Dahlén, S.-E.; Fritsch, J.; Hedqvist, P., Eds.; Raven: New York, **1990**; Vol. 20, pp. 241-249.

43. Schwartzman, M.; Ferreri, N.R.; Carroll, M.A.; Songu-Mize, E.; McGiff, J.C. *Nature* **1985**, *314*, 620-622.

44. Cunningham, F.M.; Woolard, P.M. *Prostaglandins* **1987**, *34*, 71-78.

45. Marnett, L.J. in *Biological Oxidation Systems*, Reddy, C.C.; Hamilton, G.A.; Madyastha, K.M., Eds.; Academic: San Diego, **1990**; Vol. II, p. 637-655.

46. Nelson, N.A.; Kelly, R.C.; Johnson, R.A. *Chem. Eng. News* **1982**, *60*, 30-45.

47. Shimuzu, T.; Wolfe, L.S. *J. Neurochem.* **1990**, *55*, 1-14.

48. Gardiner, P.J. in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Samuelsson, B.; Dahlén, S.-E.; Fritsch, J.; Hedqvist, P., Eds.; Raven: New York, **1990**; Vol. 20, pp. 110-118.

49. Corey, E.J.; Ensley, H.E.; Hamberg, M.; Samuelsson, B. *J. Chem. Soc. Chem. Commun.* **1975**, 277-278.

50. Brash, A.R.; Baertschi, S.W.; Ingram, C.D.; Harris, T.M. *J. Biol. Chem.* **1987**, *262*, 15829-15839.

51. Corey, E.J.; Matsuda, S.P.T. *Tetrahedron Lett.* **1987**, *28*, 4247-4250.

52. Gerwick, W.H.; Bernart, M.W.; Moghaddam, M.F.; Jiang, Z.D.; Solem, M.L.; Nagle, D.G. *Hydrobiologia* **1990**, *204/205*, 621-628.

53. Gerwick, W.H.; Bernart, M.W. in *Advances in Marine Biotechnology: Pharmaceutical and Bioactive Natural Products*, Zaborsky, O.R.; Attaway, D.H., Eds.; Plenum: New York, in press.

54. Theorell, H.; Holman, R.T.; Akeson, A. *Acta. Chem. Scand.* **1947**, *1*, 571-576.

55. Hamberg, M.; Samuelsson, B. *Proc. Nat. Acad. Sci. USA* **1974**, *71*, 3400-3404.

56. Nugteren, D.H. *Biochim. Biophys. Acta* **1975**, *380*, 299-307.

57. Corey, E.J.; Nagata, R. *J. Am. Chem. Soc.* **1987**, *109*, 8107-8108.

58. Schewe, T.; Rapoport, S.M.; Kühn, H. *Adv. Enzymol.* **1986**, *58*, 191-272.

59. Samuelsson, B.; Dahlen, S.; Lindgren, J.A.; Rouzer, C.A.; Serhan, C.N. *Science* **1987**, *237*, 1171-1176.

60. Greaves, M.W.; Camp, R.D.R. *Arch. Dermatol. Res.* **1988**, *280 [Suppl.]*, S33-S41.

61. Brash, A.R. *Circulation* **1985**, *72*, 702-707.

62. Piomelli, D.; Greengard, P. *Trends Pharmacol. Sci.* **1991**, *11*, 367-373.

63. Groß, E.; Ruzicka, T.; Mauch, C.; Krieg, T. *Prostaglandins* **1988**, *36*, 49-58.

64. Stanley-Samuelson, D.W. *Biol. Bull.* **1987**, *173*, 92-109.

65. Weinheimer, A.J.; Spraggins, R.L. *Tetrahedron Lett.* **1969**, *59*, 5185-5188.

66. Light, R.J.; Samuelsson, B. *Eur. J. Biochem.* **1972**, *28*, 232-240.

67. Schneider, Hamilton; R.D. Rhuland, L. E. *J. Am. Chem. Soc.* **1972**, *94*, 2122-2123.

68. Campbell, W.B. in *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A.G.; Rall, T.W.; Nies, A.S.; Taylor, P., Eds. Pergamon: New York, **1990**; Chapter 24.

69. Gerhart, D.J. *Mar. Ecol. Prog. Ser.* **1984**, *19*, 181-189.

70. Morse, D.E.; Kayne, M.; Tidyman, M.; Anderson, S. *Biol. Bull.* **1978**, *154*, 440-452.

71. Doerge, D.R.; Corbett, M.D. *Experientia* **1982**, *38*, 901-902.

72. Brash, A.R. *J. Am. Chem. Soc.* **1989**, *111*, 1891-1892.

73. Baertschi, S.W.; Brash, A.R.; Harris, T.W. *J. Am. Chem. Soc.* **1989**, *111*, 5003-5005.

74. Brash, A.R.; Baertschi, S.W.; Harris, T.W. in *Biological Oxidation Systems*, Reddy, C.C.; Hamilton, G.A.; Madyastha, K.M., Eds.; Academic: San Diego, **1990**; Vol. II, p. 683-693.

75. Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1982**, *23*, 5171-5174.

76. Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1983**, *24*, 1549-1552.

77. Kobayashi, M.; Yasuzawa. T.; Yoshihara, M.; Akutsu, H.; Kyogoku, Y.; Kitagawa, I. *Tetrahedron Lett.* **1982**, *23*, 5331-5334.

78. Kobayashi, M.; Yasuzawa. T.; Yoshihara, M.; Son, B.Y.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1983**, *31*, 1440-1443.

79. Munro, M.H.G.; Luibrand, R.T.; Blunt, J.W. in *Bioorganic Marine Chemistry,* Scheuer, P.J., Ed.; Springer-Verlag: Berlin, **1987**; Vol. 1, Chapter 1.

80. Iguchi, K.; Kaneta, S.; Mori, K.; Yamada, Y.; Honda, A.; Mori, Y. *Tetrahedron Lett.* **1985**, *26*, 5787-5790.

81. Iguchi, K.; Kaneta, S.; Mori, K.; Yamada, Y.; Honda, A.; Mori, Y. *J. Chem. Soc. Chem. Commun.* **1986**, 981-982.

82. Honda, A.; Mori, Y.; Iguchi, K.; Yamada, Y. *Prostaglandins* **1988**, *36*, 621-630.

83. Corey, E.J.; Lansbury, P.T.; Yamada, Y. *Tetrahedron Lett.* **1985**, *26*, 4171-4174.

84. Corey, E.J.; d'Alarcao, M.; Matsuda, S.P.T.; Lansbury, P.T. *J. Am. Chem. Soc.* **1987**, *107*, 289-290.

85. Baker, B.J., Okuda, R.K.; Yu, P.T.K.; Scheuer, P.J. *J. Am. Chem. Soc.* **1985**, *107*, 2976-2977.

86. Suzuki, M.; Morita, Y.; Yanagisawa, A.; Baker, B.J.; Scheuer, P.J., Noyori, R. *J. Org. Chem.* **1988**, *53*, 286-295.

87. Wells, R.J. *Tetrahedron Lett.* **1976**, *17*, 2637-2638.

88. Quiñoà, E.; Kho, E.; Manes, L.V.; Crews, P.; Bakus, G.J. *J. Org. Chem.* **1986**, *51*, 4260-4264.

89. Sakemi, S.; Higa, T.; Anthoni, U.; Christophersen, C. *Tetrahedron* **1987**, *43*, 263-268.

90. Molinski, T.F.; Ireland, C.M. *J. Org. Chem.* **1988**, *53*, 2103-2105.

91. Niwa, H.; Wakamatsu, K.; Yamada, K. *Tetrahedron Lett.* **1989**, *30*, 4543-4546.

92. Kigoshi, H.; Niwa, H.; Yamada, K.; Stout, T.J.; Clardy, J. *Tetrahedron Lett.* **1991**, *32*, 2427-2428.

93. Piomelli, D.; Volterra, A.; Dale, N.; Siegelbaum, S.A.; Kandel, E.R.; Schwartz, J.H.; Belardetti, F. *Nature* **1987**, *328*, 38-43.

94. Piomelli, D.; Shapiro, E.; Feinmark, S.J.; Schwartz, J.H. *J. Neurosci.* **1987**, *7*, 3675-3686.

95. Piomelli, D.; Feinmark, S.J.; Shapiro, E.; Schwartz, J.H. *J. Biol. Chem.* **1988**, *263*, 16591-16596.

96. Pace-Asciak, C.R.; Martin, J.M.; Corey, E.J. *Prog. Lip. Res.* **1986**, *25*, 625-628.

97. Derewlany, L.O.; Pace-Asciak, C.R.; Radde, I.C. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1466-1469.

98. Piomelli, D.; Shapiro, E.; Zipkin, R.; Schwartz, J.H.; Feinmark, S.J. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1721-1725.

99. Ojika, M.; Yoshida, Y.; Nakayama, Y.; Yamada, K. *Tetrahedron Lett.* **1990**, *31*, 4907-4910.

100. Cimino, G.; Spinella, A.; Sodano, G. *Tetrahedron Lett.* **1989**, *30*, 3589-3592.

101. Di Marzo, V.; Cimino, G.; Crispino, A.; Minardi, C.; Sodano, G.; Spinella, A. *Biochem. J.* **1991**, *273*, 593-600.

102. Cimino, G.; Crispino, A.; Di Marzo, V.; Sodano, G. *Experientia* **1991**, *47*, 56-60.

103. Cimino, G.; Crispino, A.; Di Marzo, V.; Spinella, A.; Sodano, G. *J. Org. Chem.* **1991**, *56*, 2907-2911.

104. Meijer, L.; Brash, A.R.; Bryant, R.W.; Ng, K.; Maclouf, J.; Sprecher, H. *J. Biol. Chem.* **1986**, *261*, 17040-17047.

105. D'Auria, M.V.; Minale, L.; Riccio, R.; Uriarte, E. *Experientia* **1988**, *44*, 719-720.

106. Hawkins, D.J.; Brash, A.R. *J. Biol. Chem.* **1987**, *262*, 7629-7634.

107. Holland, D.L.; East, J.; Gibson, K.H.; Clayton, E.; Oldfield, A. *Prostaglandins* **1984**, *29*, 1021-1029.

108. Pace-Asciak, C.R. *Prostagl. Leukotrienes Med.* **1986**, *22*, 1-9.

109. Song, W.-C.; Holland, D.L.; Gibson, K.H.; Clayton, E.; Oldfield, A. *Biochim. Biophys. Acta* **1990**, *1047*, 239-246.

110. Gregson, R.P.; Marwood, J.F.; Quinn, R.J. *Tetrahedron Lett.* **1979**, *20*, 4505-4506.

111. Quinn, R.J. in *Bioorganic Marine Chemistry*, Scheuer, P.J., Ed.; Springer-Verlag: Berlin, **1988**; Vol. 2, p. 14.

112. Fusetani, N.; Hashimoto, K. *Bull Jap. Soc. Sci. Fish.* **1984**, *50*, 465-469.

113. Jiang, Z.D.; Gerwick, W.H. *Phytochemistry* **1990**, *29*, 1433-1440.

114. Jiang, Z.D.; Gerwick, W.H. *Phytochemistry* **1991**, *30*, 1187-1190.

115. Moghaddam, M.F.; Gerwick, W.H. *Phytochemistry* **1990**, *29*, 2257-2259.

116. Croset, M.; Lagarde, M. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 878-833.

117. Turner, S.R.; Tainer, J.A.; Lynn, W.S. *Nature* **1975**, *257*, 680-681.

118. Lopez, A.; Gerwick, W.H. *Tetrahedron Lett.* **1988**, *29*, 1505-1506.

119. Moghaddam, M.F.; Gerwick, W.H.; Ballantine, D.L. *Prostaglandins* **1989**, *37*, 303-308.

120. Moghaddam, M.F.; Gerwick, W.H.; Ballantine, D.L. *J. Biol. Chem.* **1990**, *265*, 6126-6130.

121. Solem, M.L.; Jiang, Z.D.; Gerwick, W.H. *Lipids* **1989**, *24*, 256-260.

122. Lumin, S.; Falck, J.R. *Tetrahedron Lett.* **1990**, *31*, 2971-2974.

123. Nagle, D.G.; Gerwick, W.H. *Tetrahedron Lett.* **1990**, *31*, 2995-2998.

124. Guerierro, A.; D'Ambrosio, M.; Pietra, F. *Helv. Chim. Acta* **1990**, *73*, 2183-2189.

125. Biomol Catalog, Biomol Research Laboratories: Plymouth Meeting, PA, **1988**; 3rd ed.

126. Kakisawa, H.; Asari, F.; Kusimi, T.; Toma, T.; Sakurai, T; Oohusa, T.; Hara, Y.; Chihara, M. *Phytochemistry* **1988**, *27*, 731-735.

127. Barrow, R.A.; Capon, R.J. *Aust. J. Chem.* **1990**, *43*, 895-911.

128. Boland, W.; Jaenicke, L.; Müller, D.G.; Gassmann, G. *Experientia* **1987**, *43*, 466-467.

129. Kurata, K.; Taniguchi, K.; Shiraishi, K.; Hayama, N.; Tanaka, I.; Suzuki, M. *Chem. Lett.* **1989**, 267-270.

130. Cardellina, J.H.; Dalietos, D.; Marner, F.-J.; Mynderse, J.S.; Moore, R.E. *Phytochemistry* **1978**, *17*, 2091-2095.

131. Cardellina, J.H.; Marner, F.-J.; Moore, R.E. *J. Am. Chem. Soc.* **1979**, *107*, 240-242.

132. Cardellina, J.H.; Moore, R.E. *Tetrahedron* **1980**, *36*, 993-996.

133. Gerwick, W.H.; Reyes, S.; Alvarado, B. *Phytochemistry* **1987**, *26*, 1701-1704.

134. Beneytout, J.L.; Adrianarison, R.H.; Rakotoarisoa, Z.; Tixier, M. *Plant Physiol.* **1989**, *91*, 367-372.

135. Vick, B.A.; Zimmerman, D.C. in *The Biochemistry of Plants: A Comprehensive Treatise*, Stumpf, P.K., Ed.; Academic: New York, **1987**; Vol. 9, Chapter 3.

136. Anderson, J.M. in *Second Messengers in Plant Growth and Development*, Boss, W.F., Ed.; A.R. Liss: New York, **1989**; Chapter 8.

137. Zimmerman, D.C.; Vick, B.A. in *Lipid Peroxidation in Biological Systems*, Sevanian, A., Ed.; American Oil Chemist's Society: Champaign, IL, **1988**; Chapter 13.

138. Kühn, H.; Weisner, R.; Alder, L.; Schewe, T. *Eur. J. Biochem.* **1989**, *186*, 155-162.

139. Fobel, M.; Lynch, D.V.; Thompson, J.E. *Plant Physiol.* **1987**, *85*, 204-211.

140. Siedow, J.N.; Peterman, T.K. *Plant Physiol.* **1985**, *78*, 690-695.

141. Hertel, H.; Hieke, B.; Schewe, T.; Hoffmann, P. *Biochem. Physiol. Pflanzen* **1987**, *182*, 443-447.

142. Sekiya, J.; Kajiwara, T.; Munechika, K.; Hatanaka, A. *Phytochemistry* **1983**, *22*, 1867-1869.

143. Vick, B.A.; Zimmerman, D.C. *Plant Physiol.* **1989**, *90*, 125-132.

144. Zimmerman, D.C.; Coudron, C.A. *Plant Physiol.* **1979**, *63*, 536-541.

145. Masui, H.; Kondo, T.; Kojima, M. *Phytochemistry* **1989**, *28*, 2613-1615.

146. Vick, B.A.; Zimmerman, D.C. *Plant Physiol.* **1984**, *75*, 458-461.

147. Anderson, J.M.; Spilatro, S.R.; Klauer, S.F.; Franceschi, V.R. *Plant Sci.* **1989**, *62*, 45-52.

148. Hamberg, M. *Biochem. Biophys. Acta.* **1987**, *920*, 76-84.

149. Brash, A.R.; Baertschi, S.W.; Harris, T.M. *J. Biol. Chem.* **1990**, *265*, 6705-6712.

150. Song, W.-C.; Brash, A.R. *Science* **1991**, *253*, 781-784.

151. Weete, J.D. *Lipid Biochemistry of Fungi and Other Organisms*; Plenum: New York, **1980**.

152. Shechter, G.; Grossman, S. *Int. J. Biochem.* **1983**, *15*, 1295-1304.

153. Hamberg, M.; Herman, R.P.; Jacobsson U. *Biochim. Biophys. Acta* **1986**, *879*, 410-418.

154. Hamberg, M.; Herman, C.A.; Herman, R.P. *Biochim. Biophys. Acta* **1986**, *887*, 447-457.

155. Hamberg, M. *Biochim. Biophys. Acta* **1986**, *876*, 688-692.

156. Hamberg, M. *Lipids* **1989**, *24*, 249-255.

157. Champe, S.P.; Rao, P.; Chang, A. *J. Gen. Microbiol.* **1987**, *133*, 1383-1387.

158. Mazur, P.; Meyers, H.V.; Nakanishi, K.; El-Zayat, A.A.E.; Champe, S.P. *Tetrahedron Lett.* **1990**, *31*, 3837-3840.

159. Bowers, W.S.; Hoch, H.C.; Evans, P.H.; Katayama, M. *Science* **1986**, *232*, 105-106.

160. Pang, Z.; Sterner, O. *J. Org. Chem.* **1991**, *56*, 1233-1235.

161. Felle, H. *Plant Sci.* **1989**, 61, 9-15.

162. Marrè, M.T.; Albergoni, F.C.; Moroni, A.; Pugliarello, M.C. *Plant Sci.* **1989**, *61*, 21-28.

163. Erickson, K.L. in *Marine Natural Products: Chemical and Biological Perspectives*, Scheuer, P.J., Ed.; Academic: New York, **1983**; Vol. V, Chapter 4.

164. Fenical, W. *J. Phycol.* **1975**, *11*, 245-259.

165. Fenical, W.; Norris, J.N. *J. Phycol.* **1975**, *11*, 104-108.

166. Young, D.N.; Howard, B.M.; Fenical, W. *J. Phycol.* **1980**, *16*, 182-185.

167. Gerwick, W.H.; Lopez, A.; Davila, R.; Albors, R. *J. Nat. Prod.* **1987**, *50*, 1131-1135.

168. Sims, J.J.; Lin, G.H.Y.; Wing, R.M.; Fenical, W. *J. Chem. Soc. B. Chem. Commun.* **1973**, 470-471.

169. Kigoshi, H.; Shizuri, Y.; Niwa, H.; Yamada, K. *Tetrahedron* **1986**, *42*, 3781-3787.

170. San Martin, A.; Rovirosa, J.; Muñoz, O.; Chen, M.H.M.; Guneratne, R.D.; Clardy, J. *Tetrahedron Lett.* **1983**, *24*, 4063-4066.

171. Carter, G.T.; Rinehart, K.L.; Li, L.H.; Kuentzel, S.L.; Connor, J.L. *Tetrahedron Lett.* **1978**, *19*, 4479-4482.

172. Prota, G. in *Marine Natural Products: Chemical and Biological Perspectives*, Scheuer, P.J., Ed.; Academic: New York, **1980**; Vol. III, Chapter 3.

173. Christophersen, C. in *Marine Natural Products: Chemical and Biological Perspectives*, Scheuer, P.J., Ed.; Academic: New York, **1983**; Vol. V, Chapter 5.

174. Fenical, W. in *Alkaloids,: Chemical and Biological Perspectives*, Pelletier, S.W., Ed.; John Wiley and Sons: New York, **1986**; Vol. 4, Chapter 2.

175. Chapman, V.J.; Chapman, D.J. *Seaweeds and their Uses*, 3rd ed.; Chapman and Hall: London, **1980**, Chapter 2.

176. Fries, L. *J. Phycol.* **1988**, *24*, 333-337.

177. Fries, L. *Can. J. Bot.* **1984**, *62*, 1616-1620.

178. Brennan, M.R.; Erickson, K.L. *Tetrahedron Lett.* **1978**, *19*, 1637-1640.

179. Balzer, I.; Hardeland, R. *Science* **1991**, *253*, 795-797.

180. Murata, M.; Miyagawa-Kohshima, K.; Nakanishi, K.; Naya, Y. *Science* **1986**, *234*, 585-587.

181. Lake, R.J.; Brennan, M.; Blunt, J.W.; Munro, M.H.G.; Pannell, L.K. **1988**, *29*, 2255-2256.

182. Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamatsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. *Tetrahedron* **1990**, *46*, 7699-7702.

183. Wratten, S.J.; Wolfe, M.S.; Andersen, R.J.; Faulkner, D.J. *Antimicrob. Agents Chemother.* **1977**, *11*, 411-414.

184. Bano, S.; Bano, N.; Ahmad, V.U.; Shameel, M.; Amjad, S.J. *J. Nat. Prod.* **1986**, *49*, 549.

185. Kirkup, M.P.; Moore, R.E. *Tetrahedron Lett.* **1983**, *24*, 2087-2090.

186. Bernart, M.; Gerwick, W.H. *Phytochemistry* **1990**, *29*, 3697-3698.

187. Taylor, W.R. *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*; Univ. of Michigan: Ann Arbor, **1960**; p 593.

188. Aponte, N.E.; Ballantine, D.L. *Cryptogamie, Algologie* **1987**, *8*, 29-39.

189. Mendoza-Gonzales, A.C.; Mateo-Cid, L.E. *Phytologia* **1987**, *62*, 13-16.

190. Lambert, G.; Steinke, T.D.; Naidoo, Y. *S. Afr. J. Bot.* **1987**, *53*, 349-361.

191. Ballantine, D.L., personal communication.

192. Burkholder, P.R.; Burkholder, L.M.; Almodovar, L.R. *Bot. Mar.* **1960**, *2*, 149-156.

193. Ballantine, D.L.; Gerwick, W.H.; Velez, S.M.; Alexander, E.; Guevara, P. *Hydrobiologia* **1987**, *151/152*, 463-469.

194. Hornsey, I.S.; Hide, D. *Br. Phycol. J.* **1974**, *9*, 353-361.

195. Higgs, M.D., *Tetrahedron* **1981**, *37*, 4255-4258.

196. Findlay, J.A.; Patil, A.D. *Phytochemistry* **1986**, *25*, 548-550.

197. Cook, H.W. In *Biochemistry of Lipids and Membranes*; Vance, D.E.; Vance, J.E., Eds., Benjamin / Cummings: Menlo Park, **1985**; Chapter 6.

198. Corey, E.J.; De, B.; Ponder, J.W.; Berg, J.M. *Tetrahedron Lett.* **1984**, *25*, 1015-1018.

199. Higgs, M.D.; Mulheirn, L.J. *Tetrahedron* **1981**, *24*, 4259-4262.

200. Targett, N.M.; Kilcoyne, J.P.; Green, B. *J. Org. Chem.* **1979**, *44*, 4962-4964.

201. Pelletier, S.W.; Chokshi, H.P.; Desai, H.K. *J. Nat. Prod.* **1986**, *49*, 892-900.

202. Coll, J.C.; Bowden, B.F. *J. Nat. Prod.* **1986**, *49*, 934-936.

203. Alexander, L.R.; Justice, J.B.; Madden, J. *J. Chromatog.* **1985**, *342*, 1-12.

204. Rohwedder, W.K.; Cox, S.M. *Lipids* **1984**, *19*, 466-468.

205. Debry, G.; Pelletier, X. *Experientia* **1991**, *47*, 172-178.

206. Lopez, A.; Gerwick, W.H. *Lipids* **1987**, *22*, 190-194.

207. Saleh, M.H.; Rahman, F.H.A; Ibrahim, N.A.; Taha, N.M. *J. Chem. Ecol.* **1987**, *13*, 1361-1370.

208. Bernart, M.; Gerwick, W.H. *Tetrahedron Lett.* **1988**, *29*, 2015-2018.

209. Hamberg, M. *Biochem. Biophys. Acta* **1980**, *618*, 389-398.

210. Leblanc, Y.; Fitzsimmons, B.J.; Adams, J.; Perez, F.; Rokach, J. *J. Org. Chem.*, **1986**, *51*, 789-793.

211. Kuhn, H.; Weisner, R.; Lankin, V.Z.; Nekrasov, A.; Alder, L.; Schewe, T. *Anal. Biochem.* **1987**, *160*, 24-34.

212. Luckner, M. *Secondary Metabolism in Microorganisms, Plants, and Animals*, 2nd ed.; Springer-Verlag: Berlin, **1984**; p.153.

213. Woolard, P.M. *Biochem. Biophys. Res. Commun.* **1986**, *136*, 169-176.

214. Boukhchache, D.; Lagarde, M. *Biochim. Biophys. Acta* **1982**, *713*, 386-392.

215. Cunningham, F.M.; Woolard, P.M.; Camp, R.D.R. *Prostaglandins* **1985**, *30*, 497-509.

216. Samuelsson, B.; Hamberg, M. *Proc. Nat. Acad. Sci. USA*, **1974**, *71*, 3400-3404.

217. Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 7865-7869.

218. Corey, E.J.; Hopkins, P.B.; Barton, A.E.; Bangerter, B.; Borgeat, P. *Tetrahedron* **1982**, *38*, 2653-2657.

219. Kitamura, S.; Shimizu, T.; Miki, I.; Izumi, T.; Kasama, T.; Sato, A.; Sano, H.; Seyama, Y. *Eur. J. Biochem.* **1988**, *176*, 725-731.

220. Tulloch, A.P.; Bergter, L. *Lipids* **1979**, *14*, 996-1002.

221. Tulloch, A.P. *Lipids* **1982**, *17*, 544-550.

222. Bergter, L.; Seidl, P.R. *Lipids* **1984**, *19*, 44-47.

223. Westlund, P.; Edenius, C.; Lindgren, J.A. *Biochem. Biophys. Acta* **1988**, *962*, 105-115.

224. Shimizu, T.; Radmark, O.; Samuelsson, B. *Proc. Nat. Acad. Sci. USA*, **1984**, *81*, 689-693.

225. Breuer, O.; Hammarstrom, S. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 667-673.

226. Ford-Hutchinson, A.W.; Bray, M.A.; Cunningham, F.M.; Davidson, E.M.; Smith, M.J.H. *Prostaglandins* **1981**, *21*, 143-152.

227. Andersen, N.H. *J. Lipid Res.* **1969**, *10*, 316-319.

228. Pace-Asciak, C.R. *Prostagl. Leukotrienes Med.* **1986**, *22*, 1-9.

229. Pierre, J.-L.; Chautemps, P.; Arnaud, P., Gey, C. *Chim. Anal. (Paris)* **1968**, *50*, 494-500.

230. Walker, I.C.; Jones, R.L.; Wilson, N.H. *Prostaglandins* **1979**, *18*, 173-178.

231. Corey, E.J; Kang, J; Laguzza, B.C.; Jones, R.L. *Tetrahedron Lett.* **1983**, *24*, 4913-4916.

232. Mercier, J.; Agoh, B. *Chem. Phys. Lipids* **1974**, *12*, 232-238.

233. Mihelich, E.D. *Tetrahedron Lett.* **1979**, *20*, 4729-4732.

234. Mercier, J.; Agoh, B. *Chem. Phys. Lipids* **1974**, *12*, 239-248.

235. Garssen, G.J.; Veldink, G.A.; Vliegenthart, J.F.G.; Boldingh, J. *Eur. J. Biochem.* **1976**, *62*, 33-36.

236. Kato, T.; Yamaguchi, Y.; Ohnuma, S.; Uyehara, T.; Namai, T.; Kodama, M.; Shiobara, Y. *J. Chem. Soc. Chem. Commun.* **1986**, 743-744.

237. Pace-Asciak, C.R. *J. Biol. Chem.* **1984**, *259*, 8332-8337.

238. Schwartzman, M.L.; Balazy, M.; Masferrer, J.; Abraham, N.G.; McGriff, J.C.; Murphy, R.C. *Proc. Nat. Acad. Sci. USA* **1987**, *84*, 8125-8129.

239. Samuelsson, B.; Funk, C.D. *J. Biol. Chem.* **1989**, *264*, 19469-19472.

240. Miller, C.C.; Yamaguchi, R.Y.; Ziboh, V.A. *Lipids* **1989**, *24*, 998-1003.

241. Sekiya, F.; Takagi, J.; Sasaki, K.; Kawajiri, K.; Kobayashi, Y.; Sato, F.; Saito, Y. *Biochim. Biophys. Acta* **1990**, *1044*, 165-168.

242. Manganaro, F.; Gaudette, Y.; Pombo-Gentile, A.; Singh, K.; Rakhit, S. *Prostaglandins* **1988**, *36*, 859-874.

243. Gabrielson, P.W.; Scagel, R.F.; Widdowson, T.B. *Keys to the Benthic Marine Algae and Seagrasses of British Columbia, Southeast Alaska, Washington, and Oregon*; Department of Botany, The University of British Columbia: Vancouver, **1989**; pp. 16-23.

244. Fritsch, F.E. *The Structure and Reproduction of the Algae*; Cambridge University: Cambridge, **1961**; Vol. 1, pp. 236-237.

245. Abbott, I.A.; Hollenberg, G.J. *Marine Algae of California*; Stanford University: Stanford, **1976**; pp. 96-99.

246. Jónsson, S. *Ann. Sci. Nat. Bot. 12^e S., T. III*, **1962**, 25-263.

247. Fan, K.-C. *Bull. Torrey Bot. Club* **1959**, *86*, 1-12.

248. Lee, R.E. *Phycology*, 2nd ed.; Cambridge University: Cambridge, **1989**; pp. 228-230.

249. Yates, K.R. Ph.D. Thesis, Oregon State University, **1990**.

250. Pollio, A.; Della Greca, M.; Monaco, P.; Pinto, G.; Previtera, L. *Biochim. Biophys. Acta* **1988**, *963*, 53-60.

251. Zimmerman, D.C.; Vick, B.A. *Lipids*, **1973**, *8*, 264-266.

252. Waaland, J.R. *Common Seaweeds of the Pacific Coast*; Pacific Search: Seattle, **1977**, p. 41-42.

253. Müller, D.G.; Jaenicke, L.; Donike, M.; Akintobi, T. *Science* **1971**, *171*, 815-816.

254. Paul, V.J.; Fenical, W. In *Bioorganic Marine Chemistry*; Scheuer, P.J., Ed.; Springer-Verlag: Berlin, **1987**, Vol. 1, p. 4.

255. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Chirality Coupling in Organic Stereochemistry*; University Science: Mill City, **1983**; 460 pp.

256. Gonella, N.C.; Nakanishi, K.; Martin, V.S.; Sharpless, K.B. *J. Am. Chem. Soc.* **1982**, *104*, 3775-3776.

257. Solem, M.L. Master's Thesis, Oregon State University, **1988**.

258. Silverstein, R.M.; Bassler, GC.; Morrill, T.C. *Spectrometric Identification of Organic Compounds*, 4th ed.; John Wiley & Sons: New York, **1981**; 442 pp.

259. Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry*, 3rd ed.; VCH Verlagsgesellschaft: Weinheim, **1987**; p. 116.

260. Borgeat, P.; Picard, S.; Vallerand, P.; Sirois, P. *Prostaglandins Med.* **1981**, *6*, 557-570.

261. Borgeat, P.; Fruteau de Laclos, B.; Picard, S.; Drapeau, J.; Vallerand, P.; Corey, E.J. *Prostaglandins* **1982**, *23*, 713-724.

262. Lee, T.H.; Mencia-Huerta, J.-M.; Shih, C.; Corey, E.J.; Lewis, R.A.; Austen, K. F. *J. Biol. Chem.* **1984**, *259*, 2383-2389.

263. Powell, W.S.; Gravelle, F. *Biochem. Biophys. Acta* **1985**, *835*, 201-211.

264. Oliw, E.H. *J. Biol. Chem.* **1989**, *264*, 17845-17853.

265. Powell, W.S.; Gravelle, F. *J. Biol. Chem.* **1990**, *265*, 9131-9139.

266. Sok, D.-E.; Kim, M.-R. *Arch. Biochem. Biophys.* **1990**, *277*, 86-93.

267. Grechkin, A.N.; Kuramshin, R.A.; Safonova, E.Y.; Yefremov, Y.J.; Latypov, S.K.; Ilyasov, A.V.; Tarchevsky, I.A. *Biochim. Biophys. Acta* **1991**, *1081*, 79-84.

268. Metzger, P.; Casadevall, E. *Tetrahedron Lett.* **1988**, *29*, 2831-2834.

269. Metzger, P.; Villarreal-Rosales, E.; Casadevall, E.; *Phytochemistry* **1991**, 185-191.

270. Gerwick, W.H. while on sabbatical leave at Karolinska Institutet, **1990**.

271. Brash, A.R.; Hawkins, D.J. In *Methods in Enzymology*; Murphy, R.C.; Fitzpatrick, F.A., Eds.; Academic: New York, **1990**; Vol. 187, pp. 187-195.

272. Bryant, R.W.; Simon, T.C.; Bailey, J.M. *J. Biol. Chem.* **1982**, *257*, 14937-14943.

273. Fruteau de Laclos, B.; Maclouf, J.; Poubelle, P.; Borgeat, P. *Prostaglandins* **1987**, *33*, 315-337.

274. Glasgow, W.C.; Harris, T.M.; Brash, A.R. *J. Biol. Chem.* **1986**, *261*, 200-204.

275. Fruteau de Laclos, B.; Borgeat, P. *Biochim. Biophys. Acta* **1988**, *958*, 424-433.

276. Brash, A.R.; Baertschi, S.W.; Harris, T.M. *J. Biol. Chem.* **1990**, *265*, 6705-6712.

277. Hamberg, M. *Chem. Phys. Lipids* **1988**, *46*, 235-243.

278. Hamberg, M.; Hughes, M.A.; *Lipids* **1988**, *23*, 469-475.

279. Hamberg, M.; Gotthammar, B. *Lipids* **1973**, *8*, 737-744.

280. Hamberg, M. *Lipids* **1975**, *10*, 87-92.

281. Dix, T.A.; Marnett. L.J. *J. Biol. Chem.* **1985**, *260*, 5351-5357.

282. Gardner, H.W. *Biochem. Biophys. Acta* **1989**, *1001*, 274-281.

283. Ohta, H.; Ida, S.; Mikami, B.; Morita, Y. *Plant Cell Physiol.* **1986**, *27*, 911-918.

284. Kato, T.; Yamaguchi, Y.; Ohnuma, S.; Uyehara, T.; Namai, T.; Kodama, M.; Shiobara, Y. *Chem. Lett.* **1986**, 577-580.

285. Kato, T.; Yamaguchi, Y.; Hirano, T.; Yokoyama, T.; Uyehara, T.; Namai, T.; Yamanaka, S.; Harada, N. *Chem. Lett.* **1984**, 409-412.

286. Hamberg, M. *Anal. Biochem.* **1971**, *43*, 515-526.

287. Funk, M.O.; Andre, J.C.; Otsuki, T. *Biochemistry* **1987**, *27*, 6880-6884.

288. Weidenfeld, H.; Knoch, F.; Koch, M. *Arch. Pharm. (Weinheim)* **1985**, *318*, 289-293.

289. Bittner, M.; Gonzalez, F.; Valdebenito, H.; Silva, M.; Paul, V.J.; Fenical, W.; Chen, M.H.M.; Clardy, J. *Tetrahedron Lett.* **1987**, *28*, 4031-4032.

290. San-Martin, A.; Rovirosa, J.; Xu, C.; Lu, H.S.M.; Clardy, J. *Tetrahedron Lett.* **1987**, *28*, 6013-6014.

291. Faulk, D.D.; Fry, A. *J. Org. Chem.* **1970**, *35*, 364-368.

292. Jackman, L.M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon Press: Oxford, **1969**, pp. 171, 223.

293. Sato, Y.; Gould, S.J.; Kohnert, R. *Tetrahedron Lett.* **1986**, *27*, 143-147.

294. Saito, Y. *Phycologia* **1982**, *21*, 299-306.

295. Wahlberg, I; Karlsson, K.; Austin, D.J.; Junker, N; Roeraade, J.; Enzell, C.R.; Johnson, W.H. *Phytochemistry* **1977**, *16*, 1233-1235.

296. Idstein, H.; Herres, W.; Schreier, P. *J. Agric. Food Chem.* **1984**, *32*, 383-389.

297. Stephenson, W.A. *Seaweed in Agriculture and Horticulture*; Faber and Faber: London, **1968**.

298. Abe, H.; Uchiyama, M.; Sato, R. *Agric. Biol. Chem.* **1972**, *36*, 2259-2260.

299. Raub, M.F.; Cardellina, J.H.; Schwede, J.G. *Phytochemistry* **1987**, *26*, 619-620.

300. BeMiller, J.N.; Colilla, W. *Phytochemistry* **1972**, *11*, 3393-3402.

301. Rouillon, R.; Gay, G.; Bernillon, J.; Favre-Bonvin, J.; Bruchet, G. *Can. J. Bot.* **1986**, *64*, 1893-1897.

302. Chowdhury, B.K.; Chakraborty, D.P. *Phytochemistry* **1971**, *10*, 481-483.

303. Powers, J.C. *J. Org. Chem.* **1968**, *33*, 2044-2050.

304. Narumiya, S.; Takai, K.; Tokuyama, T.; Noda, Y.; Ushiro, H.; Hayaishi, O. *J. Biol. Chem.* **1979**, *254*, 7007-7015.

305. Tsujii, S.; Rinehart, K.L.; Gunasekera, S.P.; Kashman, Y.; Cross, S.S.; Lui, M.S.; Pomponi, S.A.; Diaz, M.C. *J. Org. Chem.* **1988**, *53*, 5446-5453.

306. Mashkovskii, M.D.; Suvorov, N.N.; Preobrazhenskaya, M.N.; Liberman, S.S.; Orlova, L.M.; Gurovich, I.; Avrutskii, G.; Nikitina, L.A.; Balashova, E.G.; Soviet Union Patent Number 1,164,218; **1967**.

307. Bano, S.; Ahmad, V.U.; Perveen, S.; Bano, N.; Shameel, S.; Shameel, M. *Planta Med.* **1987**, *53*, 117-118.

308. Cardellina, J.H.; Nigh, D.; VanWagenen, B.C. *J. Nat. Prod.* **1986**, *49*, 1065-1067.

309. Phinney, H.K., personal communication.

310. Devys, M.; Barbier, M. *Phytochemistry* **1991**, *30*, 389-391.