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Farlowia mollis is a marine red alga that grows in the intertidal region along the coast of Oregon. We began studying the chemistry of this alga after a survey of Oregon seaweeds revealed possibly interesting chemistry and antimicrobial activity in its crude organic extract.

Over the past three years the biomedicinal potential of nearly 100 species of Oregon seaweeds has been explored in the Oregon seaweed survey. The survey screens the aqueous and organic extracts of different algal collections for antimicrobial activity, brine shrimp toxicity and interesting chemical metabolites as judged by thin layer chromatography. Farlowia mollis was one of 17 different species of algae that were identified as promising for further study.

The natural products of Farlowia mollis include three unique diol-containing fatty acids whose structures were determined by a combination of spectroscopic techniques, including 2D-NMR, mass spectrometry, and IR, UV and circular dichroic spectroscopy.

These structures are very similar to several mammalian icosanoids which possess a wide range of biological activities. We decided to test these seaweed compounds for biological activity in common with several mammalian icosanoids, specifically for their ability to stimulate superoxide anion production in human neutrophils. It appears that the "farlowdiols" are moderate inhibitors of superoxide anion production in cells stimulated with formyl-methionyl-leucyl-phenylalanine. Other biological activities of the "farlowdiols" include mild inhibition of the dog kidney Na^+/K^+ ATPase and of the enzyme 5-lipoxygenase.

The biological roles of the "farlowdiols" in the alga Farlowia mollis are not known, however, several other icosanoids have recently been isolated from other red algae, and have identical or similar structures to several biologically active mammalian icosanoids. These icosanoids may play important hormonal and bioregulatory roles in the algae, and may aid research in understanding the roles that icosanoids play in asthma, inflammation and other disease states.

Novel Icosanoids from the Red Marine Alga *Farlowia mollis*

by

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NOVEL ICOSANOIDS FROM THE RED MARINE ALGA *FARLOWIA MOLLIS*

Chapter I: Introduction

Plants have been used for millions of years to treat a spectrum of diseases and other maladies that afflict humankind.[1] Most medicines in the past have been predominantly from terrestrial plants and bacteria, leaving marine and freshwater sources virtually unexplored.[2,13] But in the last few decades there has been a surge of interest devoted to the exploration of marine natural products and their application to pharmacology and medicinal sciences.[3,4] During this time, several valuable compounds have been isolated from marine organisms with potential in treating a variety of ailments.[13,14]

Motivation for the study of the chemistry of marine organisms is provided by the need to discover new and useful molecular structures that have potential value in the treatment of diseases in humans and other animals. New chemotherapeutic drugs and antibiotics are in constant demand as a better understanding of the biochemistry and pathology of disease states is achieved, and as various disease-causing organisms develop resistance to current drug therapy. Studies indicate that marine organisms may be a richer source of medicinals than are terrestrial plants.[5,6] The valuable pharmaceutical stores of these organisms can be utilized only

by discoveries spun by the research of marine natural products chemistry.

Valuable Natural Products of Marine Origin

For thousands of years, humans have been exploring the sea for new agents to combat disease and alleviate pain.[7] Suprisingly, there are only a few pharmaceutical products available that derive from the sea; these include agar, carrageenan, alginates, protamine sulphate and cod oils. Western cultures have utilized marine algae mainly as agricultural agents either as food for domestic animals or as fertilizers for the soil.[8,21]

Several eastern cultures have employed extracts of marine algae and other organisms in their folk remedies and in many foods.[13,21] Algae have been used in some parts of the world as anthelmintic agents, as dressings on fresh cuts and wounds and for other medical applications.[21] The use of seaweeds by the Chinese in their herbal medicines dates back over 2000 years according to ancient literature.[22] Several species of seaweeds were effective in treating goiter, scrofula and dropsy for more than 20 centuries.[22] Not until the 1950's did western societies begin to recognize marine algae as potential sources of valuable biomedicinals.[9]

Several seaweeds have been found to contain potent antitumor activity.[23] The pharmacological probe,

stypolidione, which inhibits microtubulin polymerization, is an example of a valuable biochemical tool which derives from a seaweed.[14]

Common Natural Products of Marine Algae

There are both significant differences and many similarities that exist in the biosynthetic pathways of marine and terrestrial plants.[9] The common pathways leading to the terpenes, acetogenins, aromatics and alkaloids of terrestrial plants are often different in marine algae, possibly to meet the need for appropriate lines of chemical defense; an example is the halogenation of terpenes and the production of bromine-containing acetogenins and phenolic compounds.[9] Similarities between marine algae and mammals also exist in the production of biologically active compounds. Probably one of the most striking discoveries in marine algal natural products was the isolation of the prostaglandins PGE_1 and $PGF_{2\alpha}$ from the marine alga Gracilaria lichenoides.[10]

As may be expected, different types of algae are unique in their chemical metabolites. For example, the blue-green algae (cyanobacteria) commonly produce various indole alkaloids and other nitrogen-containing compounds, like unusual peptides.[11] Common metabolites of the green algae are diterpenes containing 1,4-diacetoxybutadiene moieties and

brominated aromatics.[11] The brown algae have a diversity of structural classes, and are noted for their polysaccharides and polymeric phenolics - the phlorotannins.[11] A variety of simple lipids, presumably derived from fatty acids, have been also isolated from brown algae and have been found to act as chemical messengers.[88] Also, prenylated quinones and a variety of sesquiterpenes and diterpenes have been isolated in large numbers from a variety of brown algae.[11] The red algae contain many different types of halogenated lipids; two representative lipids are the rhodophytins (7 and 8 membered ethers with a chlorine at position 6) and halogenated monoterpenes.[11] Although the non-halogenated lipids from red algae have not been studied to such an extent, some have been isolated, and have proven to have interesting biological activities.[11] Recently, from the red algae Ptilota filicna and Murrayella peridados, several novel icosanoids have been isolated with very interesting biological properties.[12,27] (also see Chapter IV in this thesis)

The Presence of Icosanoids and Other Lipids in Plants and Algae

Lipids encompass a large and diverse group of compounds with equally diverse biological properties and functions. They are present in all living organisms, and their roles include: 1) the major structural element of membranes for all

types of cells 2) energy stores 3) precursors to many vitamins, bile acids and coenzymes 4) a variety of hormonal and cell-modulating activities. Fatty acids are a class of lipid essential to both plants and animals, but there are notable differences between plant and animal fatty acids.[24] The most abundant plant unsaturated fatty acids usually contain 18 carbons, whereas animal unsaturated fatty acids usually contain 20 carbons. Plants are capable of desaturating a fatty acid at the omega 6 and the omega 3 end of the fatty acid chain, unlike most mammals. Also, plants contain other lipids with a wide variety of functional groups such as the acetylenes, allenes, cyclopropanes, furans, epoxides and ketones.[24]

The functions of fatty acids in both plants and animals are mainly as energy reservoirs in the form of triglycerides, or as membrane structures in the form of phospholipids. However, in the past twenty years some free fatty acids have been found to be associated with a variety of biochemical cascade reactions and cell-modulating activities.[15] Since the structural elucidation of the prostaglandins in the early 1960's, and the leukotrienes in the 1970's and 1980's, an explosion of research has revealed diverse biological activities in these types of fatty acid derivatives known collectively as the icosanoids. Icosanoids are compounds related to or deriving from arachidonic acid.[15,16]

The icosanoids are not produced exclusively in mammalian

systems. Several icosanoids have been isolated from marine algae and other plants, as well as from a number of primitive marine invertebrates.[10,12,17,24] Arachidonate and icosapentaenoate are found in substantial amounts in ferns, mosses and algae (up to 40% of the total fatty acid content).[24] Seasonal variations in the contents of these fatty acids have been observed in both mosses and marine algae.[24] The functions of icosanoids in plants have not been determined, but some speculation exists as to their possible roles as hormones and as gamete attractants in marine algae.[14,24]

The prostaglandins PGE_2 (1) and $PGF_{2\alpha}$ (2) (Figure I.1) have been found in the marine alga Gracilaria lichenoides. This discovery prompted speculation that algae and other plants may utilize arachidonic acid as a precursor to the cyclooxygenase-derived prostaglandins in direct analogy to the biosynthetic mechanism used by mammals.[10] $PGF_{2\alpha}$ has also been isolated from the flowering higher plant Kalanchoe blossfeldiana. Observations were made that feeding cyclooxygenase inhibitors to these plants would inhibit flowering.[24]

Other prostaglandin-like compounds have been isolated from terrestrial plants.[17,18] A prostaglandin-like C16 fatty acid (3) has been isolated from the aquatic plant, Lemna trisulca, which grows in Southern Italy.[20] Another prostaglandin-like compound, dicranenone A (4), a cyclopentene-containing C18 fatty acid, was isolated from several

Japanese mosses.[17] Some halogenated prostaglandin-like compounds have been isolated from marine organisms, and have been found to contain potent antitumor activities.[17]

Other icosanoids which have structural features in similarity to the lipoxygenase-derived leukotrienes, lipoxins, Hetes and diHetes produced by animals, have also been found in several plants, including marine algae. Lipoxygenases are widely distributed throughout the plant kingdom and produce oxidized fatty acids in a very specific manner.[24] A lipoxygenase isolated from potatoe tubers and tomato plants mediates the selective oxygenation of arachidonate to give optically active 5S-hydroperoxy-6E,8Z,11Z,14Z-icosatetraenoic acid. This hydroperoxy compound is the first intermediate "en route" to the leukotrienes. Recently, two 20 carbon fatty acids (**5**) and (**6**) containing a conjugated triene functionality in similarity to these leukotrienes were isolated from the red alga Ptilota filicina.[12] This red alga also contained another icosanoid-related compound (**7**).[26] These three compounds have demonstrated biological activities in common with LTB_4.[12,26]

Several hydroxy-fatty acids similar to the Hetes have been isolated from marine algae. 12-hydroxy-icosapentaenoic acid (**8**) was isolated from a red alga, Murrayella periclados, and was found to have biological activities in common with the platelet-derived 12-hydroxyicosatetraenoic acid.[27] 12-

HEPE (**8**) was also isolated from the red alga Laurencia hybrida.[27]

In similarity to the trihydroxy-containing lipoxins, several compounds have been isolated from plants and marine algae. Malyngic acid (**9**) was isolated from the blue-green alga, Lyngbya majuscula collected in the Caribbean.[25] A trihydroxy-containing 20 carbon fatty acid (**10**) was isolated from the fresh water sedge Eleocharis microcarpa.[24] Several trihydroxy-fatty acids, each containing a 1,2,5-trihydroxy-3E-pentaenoic group, have been isolated from the roots of Bryonia alba and a number of other plants.[10,18] These trihydroxy-containing compounds (**11**),(**12**),(**13**) and (**14**) have been found to raise muscular tone, increase blood clot retraction time, display a hypoglycemic action in diabetics and initiate other physiological responses.[18] It was found that these compounds are also produced by mammals, and are predominantly formed from the endogenous release of linoleic acid in mammalian blood vessels, leukocytes and epidermal tissue.[18]

Other fatty acids with some icosanoid features have been isolated from marine algae. A 9-methoxy 16 carbon fatty acid (**15**) was found in moderate amounts from the deep water variety of Lyngbya mayuseula.[11,25] From the same species in shallow water, a 7-methoxy 14 carbon fatty acid (**16**) was isolated.[25,99]

Mammalian Icosanoids from the Lipoxygenase Pathway

Mammalian systems contain various types of lipoxygenase enzymes that can add molecular oxygen to positions 5,12 and 15 of arachidonic acid,[28] and to a lesser extent icosapentaenoic acid,[29,30] to form an unstable hydroperoxy intermediate (Figure I.2). This precursor can go on to form an allylic epoxide, or can be hydrolyzed either enzymatically or nonenzymatically to form the hydroxy fatty acid.[28,30] Lipoxygenase products have been found in a variety of mammalian cells and body fluids, including polymorphonuclear leukocytes, macrophages, platelets, normal and psoratic epidermal tissue, lung, brain and synovial fluid.[27,31] Several of these cell types and tissues are known to contain lipoxygenase enzymes.[33] These lipoxygenase-derived icosanoids contain a wide range of potent biological activities.[34,35] Their presence in psoratic tissue and their involvement in asthma suggest that they are involved in the inflammatory response.[31,36] Other biological activities include neutrophil degranulation,[44] leukocyte chemotaxis,[38] aggregation[38] and the promotion of the hypersensitivity response.[35,39]

Structure Elucidation and Bioactivities of Some Lipoxygenase-derived Icosanoids

Studies over the past 15 years have lead to the discovery of a new group of compounds, the leukotrienes.[30] These compounds are derived from the lipoxygenation of arachidonate to form a conjugated triene functionality with various positions of oxidation. One of the most studied of these is LTB_4 **(17)**, a major product formed in mammalian polymorphonuclear leukocytes (PMNLs). The biological activities of LTB_4 include potent leukocyte chemotactic properties, stimulation of neutrophil aggregation and enzyme secretions and enhancement of superoxide anion production.[37,38]

The initial isolation of LTB_4 was made from rabbit PMNLs, and was later found in human and other mammalian PMNLs.[30] Rabbit PMNLs were incubated with radioactive arachidonic acid, and the products formed were isolated using silicic acid chromatography and reverse phase HPLC.[40] Several derivatives, including the methyl silyl derivative, were further analyzed by GC/MS. This allowed the determination of the location of the hydroxyl groups at positions 5 and 12 in the 20 carbon fatty acid derivative.[40] The ultraviolet spectrum showed three characteristic absorption bands between 261-281nm, indicating the presence of a conjugated triene functionality. The positions of the double bonds were

determined by oxidative ozonolysis. The stereochemistries of the double bonds were determined by direct chemical synthesis of LTB_4 and its isomers, and by comparing their retention times by GC/MS.[40] The stereochemistries of the hydroxyl groups were determined by converting LTB_4 into the methoxycarbonyl methyl ester derivative, ozonolysis and analysis by GC/MS of the products formed. By comparing their retention times to standard dimethylmalate and dimethyl-2-hydroxyadipate, the stereochemistries at positions 5 and 12 were assigned as (S) and (R) respectively.[40] Steric analysis and direct chemical synthesis confirmed the stereochemical assignments.[30] Thus, the structure of LTB_4 was determined as 5S,12R-dihydroxy-6(Z),8(E),10(E),14(Z)-icosatetraenoic acid. Several of its isomers were purified as minor components and are believed to be formed by nonenzymatic hydrolysis of an intermediate epoxy precursor.[40]

The proposed biosynthetic pathway (Figure 1.3) is supported by the following biosynthetic studies.[30] After incubating rabbit PMNLs with arachidonic acid under an atmosphere enriched with $^{18}O_2$, the ^{18}OH-fatty acid at position 5 could be isolated. Incubation of PMNLs with $H_2{}^{18}O$ enriched buffer lead to the isolation of the radiolabelled hydroxy group at C-12.[30] Additional studies revealed the presence of a highly unstable intermediate, LTA_4(**18**), whose epoxide at positions 5,6 derives from molecular O_2.[30]

Several other leukotrienes have been isolated and structurally identified. Of particular biological interest

are the mixture of leukotrienes known as the slow releasing substance of anaphylaxis (SRS-A). The initial discovery of SRS-A was made in 1938; the substance was found in perfusates of dog lungs after exposure to snake venom.[30] The isolation and structural identification of LTC_4(**19**), a component of SRS-A produced in murine mastocytoma cells, was made by Samuelsson and coworkers in the early 1970's.[30] Its biological activities include a 200 fold greater potency than histamine in contracting parenchymal strips of guinea pig lung, an in-vitro system for studying small bronchial airways.[35] In addition, LTC_4 was found to decrease arterial blood pressure and airway conduction along with its more active isomer, LTD_4(**20**).[35]

Although the chemotactic activities of LTB_4 have not been matched by other lipoxygenase-derived fatty acids, several monohydroxy-icosatetraenoic acids (HETEs) have demonstrated in vitro leukocyte-directing activities.[35] The chemotactic responses elicited by these compounds are similar in magnitude to those evoked by the chemotactic peptide C5a, the minor fragment of the fifth component of human complement.[30] The order of potency of these HETEs is as follows: 5-HETE(**21**) much greater than 8-HETE(**22**) equal to 9-HETE(**23**) greater than 11-HETE(**24**) equal to 12-HETE(**25**) greater than 15-HETE(**26**).[35] In addition, both LTB_4 and 5-HETE have demonstrated an enhancement of the expression of the C3b receptor on human neutrophils and eosinophils. This receptor has been shown to act synergistically with the receptor for

the Fc portion of IgG to enhance adherence and phagocytosis of serum opsonized micro-organisms.[41]

A closely related series of lipoxygenase-derived fatty acids have been discovered recently and structurally identified, and their trivial names given as lipoxin A, (27) and lipoxin B, (28).[42,43] These compounds were first identified as the polar products of metabolism in mixed human leukocytes after incubation with radioactive 15-HETE.[43] In addition to these products, several others are formed from the metabolism of 15-HETE in these cells: 8,15-DHETE (29), 14,15-DHETE (30), 5,15-DHETE (31), 11,14,15-THETE (32) and 11,12,15-THETE (33).[30] One biological activity of the lipoxins is the inhibition of target cell lysis by human natural killer cells. Lipoxin A can also provoke contractions of parenchymal strips and stimulate microvascular changes in guinea pigs.[43] Lipoxin A has also been found to stimulate aggregation and the production of superoxide anion without provoking the release of granular enzymes in human neutrophils.[42] Stimulation of neutrophil activities is common amongst the lipoxygenase-derived icosanoids. The DHETEs have also been found to be enhancers of superoxide anion production by human neutrophils in the presence of the chemotactic peptide, formyl-methionyl-leucyl-phenylalanine (fMLP).[30,44] FMLP is a synthetic peptide that has been derived by analogy to leukocyte chemoattractants produced by bacteria.[45] Inhibition of superoxide production by 14,15-DHETE in the presence of the stimulator LTB_4 has

also been reported.[46]

The Neutrophil

Several lipoxygenase-derived icosanoids are synthesized by mammalian polymorphonuclear leukocytes and appear to effect these cells' activities. In particular, the neutrophil appears to be significantly affected by the presence of icosanoids. Mammalian neutrophils are known to synthesis LTB_4 and other icosanoids, and these fatty acids have been found to exert many biological responses in these cells.[48,47] These responses include chemotaxis,[34] degranulation,[49] aggregation[49] and the production of superoxide anion.[43,44] Receptor sites for LTB_4 have been identified on the neutrophil,[50] and it is possible that receptors for other icosanoids will be detected as the mechanisms of these interactions are further uncovered.

White cells that contain segmented nuclei are known as polymorphonuclear leukocytes, or PMNLs.[51] This class of cells includes the basophils, the eosinophils and the neutrophils.[51] Of these three cell types, the neutrophil usually makes up about 95% or more of the total cell population in humans.[51]

The principal function of the neutrophil is to combat bacterial invasion and infection.[51] This cell contains several different types of granules of different sizes and shapes and with different granular contents. The enzymes

contained in these granules include elastases, alpha and beta glucosidases, myeloperoxidases and other degradatory enzymes.[51,52] Also, the plasma membrane of neutrophils contain oxidase enzymes that are capable of producing highly reactive oxygen species and releasing them to destroy invading microorganisms.[53,54] All of these enzymes allow the neutrophil to be an effective antimicrobial agent. Also, the neutrophil is capable of directing itself to the site of infection *via* chemotaxis.

In vitro assays for neutrophil chemotaxis and degranulatory responses, aggregation and the production of superoxide anion involve the isolation of the cells from whole blood, or from the spleen of a laboratory animal. Neutrophils are very delicate cells and are easily damaged by rough handling and by chemical impurities.[55] Isolated neutrophils do not store well and may become inactive after several hours following isolation.[55] When isolating human neutrophils, approximately 100ml of whole blood will yield about 10^8 cells. The half-life of a circulating neutrophil is very short, only about 7 hours.[56]

Assays for Neutrophil Chemotaxis

Chemotaxis by neutrophils involves several intracellular

and extracellular factors.[57] A variety of substances have been found to stimulate neutrophil chemotaxis. These include several oligopeptides produced by bacteria, C5a, fMLP and also the icosanoids produced by the neutrophil itself; LTB_4, several HETEs and some prostaglandins. Surface receptors for a number of these substances have been identified.[52,57] The mechanism for the chemotactic response is believed to involve a depolarization of the membrane following membrane recognition by the chemotaxin.[58] It is likely that the rise in intracellular Ca^{+2} and Na^{+} from outside the cell, or from intracellular stores of Ca^{+2}, induces a reorganization and reorientation of the cytoskeleton and activates the contractile process leading to polarized contractions in the direction of a chemotactic gradient.[57]

The evaluation of chemotaxis is usually done using a Boyden chamber. The chamber contains a membrane filter which can be transversed by the cells in response to a chemotactic gradient.[59,60,61] The cells are introduced on top of the membrane, in the upper chamber of the Boyden chamber, while the chemotactic agent is introduced into the lower chamber. The cells are incubated at 37°C for 1-3 hours, and the membrane is removed and stained. The number of cells that have passed through the membrane in the direction of the chemotactic agent are counted using a light microscope. An alternative to the microscopic count is the use of a spectrometer to count the cells that have passed through the

filter. The cells that have migrated towards the chemotaxin are stained and their colorimetric absorbances measured.[62]

Chemotaxis under agarose plates can be done as an alternative to the membrane filter assay.[59] In addition, collagen and fibrin gels have also been employed to measure the chemotaxis response.[63] The cells are applied into tiny chambers in the center of the gel, and the chemotaxin and control substance are applied on opposite sides of the cells. The cells are incubated at 37°C for 1-3 hours, then are stained and measured for chemotaxis using a light microscope. Time-lapse photography can be used to observe the continuing chemotaxis response.

Neutrophil Degranulation and Superoxide Anion Production

The movement of the neutrophil to the site of infection is only the first stage of its defense against bacterial invasion. Once recruited, the cell has the ability to destroy the invading organism by phagocytosis, followed by digestion with degradatory enzymes and highly reactive oxygen species. When a bacterium becomes phagocytosed by a neutrophil, it then becomes enclosed in a vacuole bound by a membrane that was originally located on the surface of the neutrophil, a process known as endocytosis. The membrane may contain the enzyme NADPH oxidase, which can generate strong oxidizing agents that begin to destroy the encapsuled microorganism.[64] Granules in the cytoplasm of the neutrophil

begin to fuse with the phagocytic vacuole seconds after phagocytosis, and begin releasing different granular enzymes onto the surface of the phagocytosed bacterium. Lysozymes hydrolyze the mucopeptides found in the bacterial wall, lactoferrins absorb essential iron from the organism, and myeloperoxidases can use hydrogen peroxides in the presence of I^- and Cl^- to form highly reactive, bacteriacidal agents.

A variety of compounds have been shown to stimulate the oxidative metabolism and release of granular enzymes of neutrophils *in vitro*. Some of them include fMLP and other formylated peptides, phorbol myristate acetate, cytochalasin B, serum-coated zymosan, and several lipoxygenase-derived fatty acids, including LTB_4 and lipoxin A.[66,65] Several of these agents promote chemotaxis of neutrophils as well as degranulation and superoxide production.

One of the most widely employed chemotactic and degranulatory agents is the synthetic formylated peptide, fMLP. Its biological properties include the stimulation of leukocyte chemotaxis, aggregation, degranulation and superoxide production in neutrophils. The peptide is often used as a model chemotactic agent.

Neutrophil activation by fMLP is mediated through plasma membrane receptors. Binding to these receptors stimulates membrane depolarization, and an increase in intracellular Ca^{+2} and fluxes in Na^+ and K^+.[66] It is believed that the increase in Ca^{+2} is mainly from intracellular stores, though the external medium may also provide the ion.[66] The Ca^{+2}

released into the cytoplasm is then taken up by calmodulin and other Ca^{+2}-sensitive proteins, triggering local physiological responses.

Arachidonic acid and its metabolites also influence neutrophil activation. Mobilization and metabolism of arachidonate are involved in neutrophil activities. The involvement of several icosanoids in mediating Ca^{+2} homeostasis has been observed.[66] Increases in the influx of intracellular Ca^{+2} may mediate the activation of phospholipase A_2 which liberates arachidonic acid from phospholipids in the cell membrane. Increases in 5-HETE, LTB_4 and other icosanoids in the cytosol can be observed after stimulation with fMLP.[68,67] It is possible that arachidonate metabolites may escape from the cell and participate in the activation and/or recruitment of other nearby cells.[66] They may act as calcium ionophores or through membrane receptors that are associated with an existing ion conductance channel protein. Evidence supports the latter conclusion at physiologically relevent concentrations of the metabolites.[50] At higher concentrations (10^{-6}M and greater), these mediators may act operationally as calcium ionophores.[66]

There appears to be a cyclic effect between Ca^{+2} and the production of arachidonate metabolites. The binding of fMLP triggers the influx of Ca^{+2}, which mediates the activation of

the Ca^{+2}-dependent phospholipase A_2 and 5-lipoxygenase. This promotes the release of arachidonate and the conversion to lipoxygenase-derived icosanoids. These fatty acid metabolites are released from the cell and act on other cells, mediating the influx of more Ca^{+2} into the cytoplasm (Figure I.4) upon binding to membrane receptors.

It is interesting to point out that various non-oxygenated fatty acids have also been found to affect the activity of neutrophils.[69,70] Both unsaturated and saturated fatty acids have the ability to stimulate the production of superoxide anion at micromolar concentrations.[70] It appears that in the presence of Ca^{+2} (between 0.3 and 0.6 micromolar), fatty acid-induced superoxide anion production by neutrophils is partially inhibited. The inhibitory effects of Ca^{+2} are felt to be due to the ionic interaction between the carboxyl group of the fatty acid and the Ca^{+2}, thus preventing ionic interactions between the fatty acid and the neutrophil membrane.[69]

The role of icosanoids in degranulation and in oxidative metabolism of neutrophils are not fully understood. In rat neutrophils, several 5-lipoxygenase inhibitors were employed to study the role of icosanoid production in the activity of the neutrophil's response to various secretogogues.[71] These inhibitors prevented degranulation and superoxide anion production by cells exposed to the secretagogues fMLP, Ca^{+2} ionophore A23187 and phorbal myristate acetate. This

suggests that the lipoxygenase-derived icosanoids may be important in mediating these responses in the cells.[71] It may be important to note that in rat neutrophils, fMLP, while stimulating the degranulatory response, does not promote the production of superoxide as in human neutrophils.[71] Inhibitory effects on the oxidative metabolism of PMNLs has been seen in the presence of the cyclooxygenase-derived icosanoids, PGE_1 and PGI_2 .[72] Both of these icosanoids have been shown to inhibit the acute inflammatory response in vivo. PGE_1 has also been found to suppress the release of endogenously formed LTB_4.[72]

Other studies using human neutrophils have found that the concentrations of 5-lipoxygenase inhibitors that are necessary to inhibit degranulation were significantly higher than the concentrations necessary to inhibit the production of LTB_4, 5-HETE and other icosanoids.[67,68] Contrary to other studies using rat neutrophils,[71] this suggests that in human neutrophils, lipoxygenase-derived icosanoids do not mediate intracellular neutrophil degranulation in the presence of fMLP or Ca^{+2} ionophore. It was also found that much lower concentrations of fMLP could elicit superoxide anion release, chemotaxis and degranulation than those necessary to stimulate the production of LTB_4.[67] These results may suggest that lipoxygenase products, while being important in mediating neutrophil recruitment and other extracellular response, may not be involved directly as intracellular

mediators. The observed release of LTB_4 from these cells in response to the stimulus fMLP may be a mechanism for amplifying cell accumulation at inflammatory sites.[67]

LTB_4 and other icosanoids appear to be important in cell-cell interactions not only among neutrophils, but also among other types of cells, and between neutrophils and platelets.[73] In vitro assays using isolated platelets and neutrophils have demonstrated that 12-HETE produced by platelet-derived lipoxygenases can serve as precursor to several DHETEs produced by neutrophils. In addition, 5-HETE produced by neutrophils was utilized by platelets in the synthesis of DHETEs and various THETEs with a range of biological activities and potencies. This phenomenon of cell-cell interaction via the lipoxygenase pathway suggests a role in hemostasis, thrombosis and inflammation.[73]

It appears that degranulation, superoxide production and aggregation are separate responses in human neutrophils that do not necessarily occur simultaneously.[74] In an experiment which employed an fMLP-butoxycarbonyl fLPLP agonist-antagonist system to allow the experimenters to systematically vary the time of agonist-receptor binding, it was found that different responses required different lengths of time of binding by fMLP.[75] Release of granular enzymes required a finite receptor occupancy by the agonist. After 10 seconds of binding, the cells were fully committed to the degranulatory response. Increases in cytosolic Ca^{+2} were also demonstrated to require only 2 seconds of receptor

occupancy by fMLP for an optimal response. Conversely, superoxide release and aggregation required continuous occupancy of the receptor by fMLP to initiate and maintain these responses. It is believed that these later two responses may require an additional factor besides Ca^{+2} to elicit these activities in human neutrophils. Some evidence exists that phospho-inositol turnover and the activation of a protein kinase may play an essential role in the activation of NADPH oxidase.[75] It is possible that the stimulus fMLP may require additional factors besides Ca^{+2} to maintain the superoxide-stimulating response.

Studies have found a correlation between the production of icosanoids with the release of granular enzymes, but not with the production of superoxide by rat neutrophils.[76] This may suggest that enzyme secretions and superoxide production are mediated by two fundamentally different intracellular pathways, although these responses derive from a common agonist-receptor binding site on the cell membrane of rat neutrophils.[76]

Evidence also exists for the separation of the chemotactic and phagocytic responses.[54] The ability of fMLP and other chemotactic peptides to stimulate the PMNL's oxidative metabolism and degranulatory response would theoretically allow for premature release of reactive metabolites by the cells responding to a chemotactic gradient.[54] This would compromise the effectiveness of the neutrophil's ability to combat bacteria. It appears that

this does not happen; instead, the cells become down-regulated in the presence of chemotactic concentrations of fMLP so that little or no superoxide is produced upon exposure to higher concentrations of the peptide. This down-regulation phenomenon does not compromise oxidative initiation by other stimulators of superoxide production, and is reversible under conditions that allow dissociation of fMLP from the receptor.[54]

It is becoming increasingly evident that neutrophils are not a completely homogeneous group of cells, but that some variability does exist which could affect their ability to respond to different stimulators.[77,78] Heterogeneity has been found in the oxidative metabolism, chemotaxis and degranulatory responses of neutrophils. Whether it is a function of their age, or if the heterogeneity reflects true subpopulations of cells is not known. It appears that the enzyme responsible for superoxide production, NADPH oxidase, may be found in two different locations in neutrophils, with some cells containing the enzyme at only one of these sites, either in the plasma membrane or in the cytosol.[53] This may explain the variability observed in the oxidative metabolism of some individuals whose cells do not respond to stimuli that interact at cell receptors, like fMLP, while responding to other stimuli, like PMA, that interacts intracellularly, probably at the site of the enzyme superoxide dismutase.[53] It appears that the older the cell is (the longer it has been isolated from whole blood) the greater is the amount of

superoxide production in response to fMLP although only the activities of isolated cells from 0 to 5 hours were reported in the study.[78]

Summary of Neutrophil Activity

Neutrophils are antimicrobial cells with the ability to move towards sites of infection, aggregate, phagocytose and produce highly reactive metabolites that destroy microorganisms. A variety of compounds have been found to stimulate these responses in neutrophils.[37,65,66] The chemotactic peptide, fMLP, may function like the formylated peptides secreted by bacteria that stimulate neutrophil chemotaxis, superoxide production and enzyme secretions. It appears that these neutrophil responses are distinct and separable.[54]

The binding of fMLP to membrane receptors triggers an increase in cytosolic Ca^{+2} from intracellular stores, and allows the ion to bind with calmodulin and Ca^{+2}-requiring enzymes to activate a response.[66] Also, the binding of fMLP may trigger the production of LTB_4 and other lipoxygenase-derived icosanoids.[67] Some of these fatty acid derivatives, particularly LTB_4, have potent chemotactic properties and can stimulate or amplify the production of superoxide with or without fMLP present.[79] It may be that these arachidonate metabolites play a role in the recruitment of other

neutrophils and other cell-cell interactions in response to stimuli, but they do not appear to be the sole mediators of intracellular activity in human neutrophils.[67,68] The ability of these lipoxygenase products to enhance the formation of superoxide anion by leukocytes in response to fMLP suggests that these compounds may act primarily as modulators of biological activities induced by other agents rather than as primary stimuli.[79]

Finally, it appears that neutrophils are heterogeneous cells in many respects, including their oxidative metabolism, chemotactic activities and mechanism for binding of fMLP.[78,53] Some differences in their responses have been correlated with the length of time they are in isolation.[54]

Methods for Evaluating Neutrophil Degranulation and Aggregation

Neutrophil degranulation can be measured either as an intra-cellular or exocytic event.[65] For exocytic degranulation, the fungal metabolite cytochalasin B is often used as an inhibitor of phagocytosis. This agent prevents microtubulin polymerization and allows the granular contents to be expelled outside the cell.[65] Some enzymes that are commonly measured in the assay include myeloperoxidase, elastase, lysozyme and beta-glucuronidase.[80] Measurements are usually made spectrophotometrically, either by a continuous assay or a fixed time assay.

Neutrophil aggregation is commonly measured using a device known as an aggregometer. This instrument measures changes in light transmission. The cells are usually stirred continuously throughout the assay and are exposed to cytochalasin B before exposure to the stimulus.[43,74] Aggregation can be measured either by a continuous assay or a fixed time assay.

Methods for Measuring the Oxidative Metabolism of Neutrophils

Several highly reactive oxygen species are generated by neutrophils in response to a stimulus.[64] Singlet oxygen is generated during the respiratory burst and can be detected as chemiluminescence. The spontaneous decay of this unstable oxygen species can be measured in a liquid scintillation counter.

Superoxide production can be determined by measuring the quantity of O_2^- produced over a defined time interval, or by measuring its continuous production. Several assays have been designed to measure the generation of superoxide spectrophotometrically. It can be measured by its reduction of the yellow dye nitroblue tetrazolium (NBT) to a blue insoluble product.[64] More commonly, the reduction of cytochrome C is used to measure directly the amount of superoxide being produced.[53,65] Cytochrome C does not penetrate the cell, so only that fraction of superoxide released into the media is measured. Superoxide dismutase,

which converts superoxide into hydrogen peroxide, is added to the control to distinguish only those species of superoxide reducing the cytochrome from any other reducing agents in the media. Cytochalasin B is often added to the reaction mixture to reduce the lag time between stimulus and neutrophil response. This agent can potentiate the response of various stimuli, including fMLP.[81] Cytochalasin B is also capable of independently stimulating superoxide production in neutrophils, so that the appropriate control should be incorporated into the experiment.

Oxygen uptake by neutrophils following exposure to a stimulus can also be measured.[53] Measurements are usually done using an oxygen electrode.

The Role of Icosanoids in Plants

It has been shown in mammalian systems that icosanoids play diverse biological roles.[35,39,44] Many *in vitro* and *in vivo* assays have demonstrated their biological effects. However, in plants the functions of these fatty acid derivatives are not as clearly understood.[82,83]

The existence of plant lipoxygenases has been known for many years. In fact, a lipoxygenase from soybeans was one of the first enzymes ever isolated in crystalline form (1947).[82] It appears that this type of enzyme can be found in all types of plants and plant parts, including legume seeds, cereals

grains, in leaves and stems and in both higher and lower plants including the algae.[82,83] Both plant and animal lipoxygenases show the same substrate specificity. Both enzymes oxygenate at the cis,cis-1,4 pentadiene moiety of a polyunsaturated fatty acid. [82,83,84] Although most lipoxygenases produce peroxy-fatty acids with the S-configuration, some enzymes have been reported that favor the production of the R-configuration.[85] Also, most plant and animal lipoxygenases have their specificity for the omega terminus of a fatty acid. For instance, lipoxygenase-1 from soybeans, will oxygenate a C-18 fatty acid at position 13 and a C-20 fatty acid at position 15 (omega 6). However, the isolation of a lipoxygenase from wheat has recently been reported with its specificity for the carboxy terminus of a fatty acid.[86] Double dioxygenation of a single fatty acid by a single enzyme is common in both plants and animals.[87]

The product of lipoxygenation is a hydroperoxy-fatty acid containing a cis,trans conjugated diene system adjacent to the position of oxygenation.[83] This product can either be enzymatically or nonenzymatically reduced to the corresponding hydroxy-fatty acid. Plant lipoxygenases have also been reported to react with different plant pigments, and can catalyze their oxidation, or bleaching.[82] The oxidation of both chlorophyll and carotenoids by lipoxygenases has also been observed.[82]

The physiological role of plant lipoxygenases is not fully understood. It has been suggested that these enzymes

are involved in the initial steps in the formation of conjugated triene lipids.[82] These types of compounds have many physiological effects in plants, including chemotactic activities as sex pheromones in brown algae.[88] The formation of these trienes may be from an unstable hydroperoxy intermediate that becomes dehydrated.

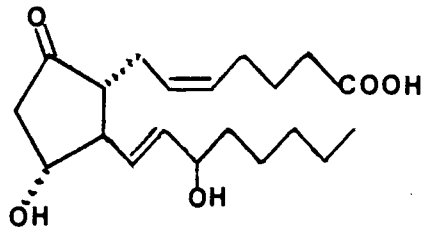
In higher plants, lipoxygenases may be involved in the formation of polymerized oxidized lipids.[82] The structural polymers, suberin and cutin, are derived from the lipoxygenase products, oxidized fatty acids joined by oxygen linkages.

It is known that lipoxygenase activity varies at different stages of the life cycle in plants.[82,83] This may suggest an involvement in different plant life-stages, like germination and senescence. It has been proposed that lipoxygenase might play a role in maintaining a low O_2 tension in seeds at certain physiological stages.[82] It is also known that lipoxygenase activity increases during senescence. It has been reported that the phytohormone cytokinin significantly lowers lipoxygenase activity during senescence.[89] This regulatory activity of cytokinin might explain its ability to inhibit plant senescence. It is suggested that this mode of action might involve the scavaging of free radicals.[89] It is interesting to note that algae exhibit variations in their fatty acid content and degree of unsaturation at different developmental stages.[90]

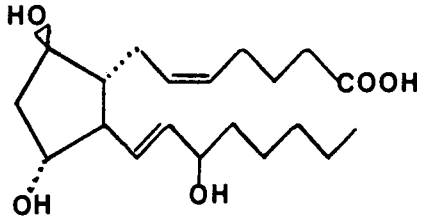
The elucidation of the structure and biosynthesis of

traumatine has revealed another possible role of lipoxygenase. This compound, 12-oxo-10E dodecenic acid, has a 13-hydroperoxy linolenic acid precursor formed from lipoxygenation. Traumatine is formed in injured plant tissue, and is believed to accelerate the division and differentiation of plant cells. Lipoxygenase activity is also known to increase in damaged plant tissues.[82,83]

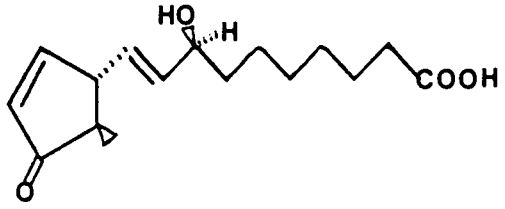
Although several lipoxygenase enzymes and their products have been identified in plants, their biological significance can only be postulated. Ecological studies may be useful in determining the environmental factors that may enhance the production of lipoxygenase products. A better understanding of the physiological stresses in plants might explain the role of these lipoxygenase products as possible biological modulators.



(1) PGE_2[10]



(2) $PGF_{2\alpha}$[10]



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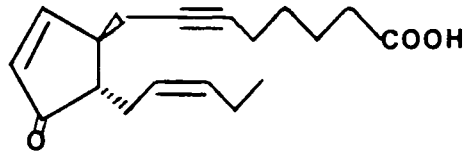
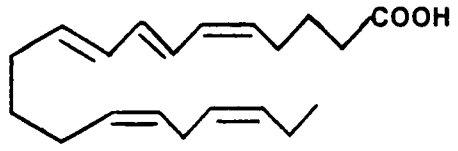
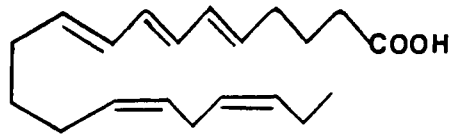


(4) dicranenone[17]

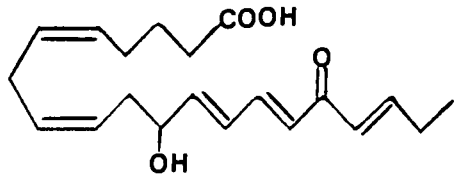


(5) 5(Z),7(E),9(E),14(Z), 17(Z)-icosapentaenoic acid[12]

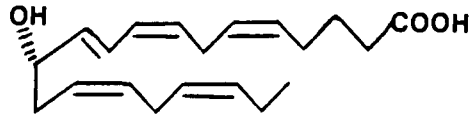
Figure I.1 Structures of different icosanoids deriving from both plants and animals



(6) 5(E),7(E),9(E),14(Z), 17(Z)-icosapentaenoic acid[12]

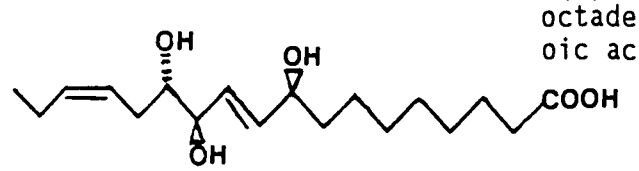


(7) 11-hydroxy-16-oxo-5(Z), 8(Z),12(E),14(E),17(E)-icosapentaenoic acid[26]



(8) 12(S)hydroxy-5(Z),8(Z), 10(E),14(Z),17(Z)-icosapentaenoic acid[27]

(9) 9(S),12(R),13(S)trihydroxy octadeca-10(E),15(Z)-dienoic acid[25]



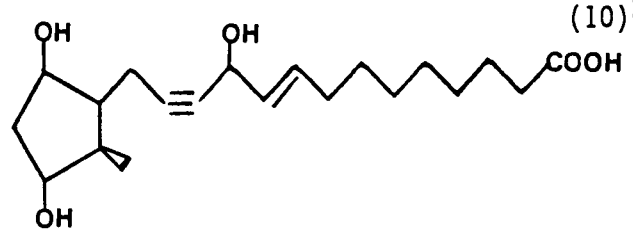


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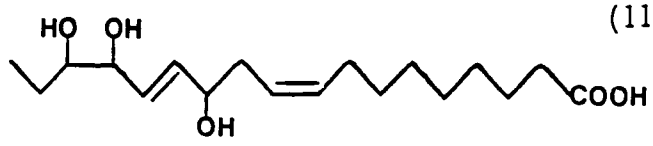
Figure I.1 continued



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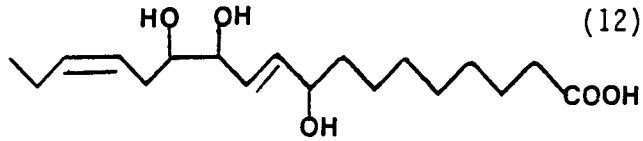

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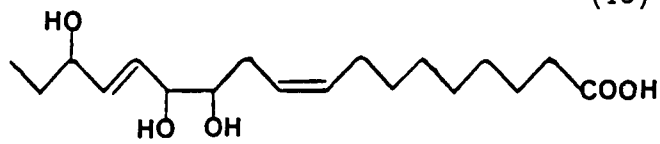

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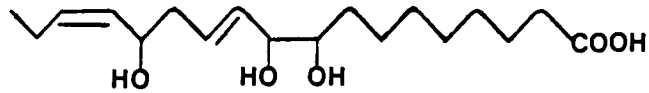

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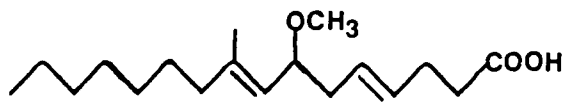
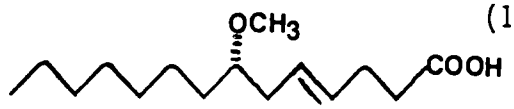
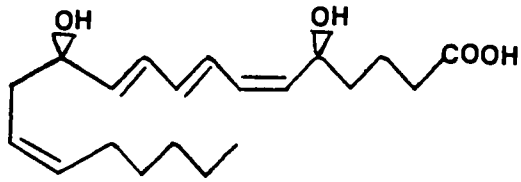


(15) 7-methoxy-9-methyl-hexadeca-4(E),8(E)-dienoic acid[25]

Figure I.1 continued

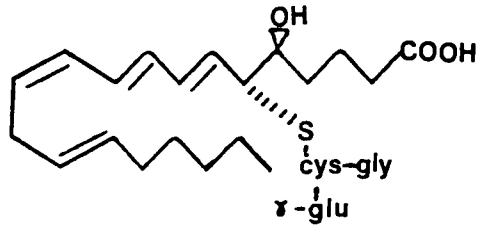


(16) 7(S)-methoxytetradeca-4(E)-enoic acid[25]

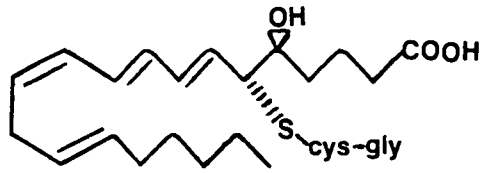


(17) LTB_4[15]
5(S),12(R)dihydroxy-6(Z),8(E),10(E),14(Z)-icosatetraenoic acid

(18) LTA_4[15]
5,6-oxido-7(E),9(E),11(Z),14(E)-icosatetraenoic acid

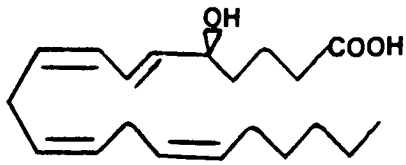


(19) LTC_4[15]
5(S)-hydroxy-6(R)-glutamyl-cysteinylglycyl 7(E),9(E),11(Z),14(E)-icosatetraenoic acid

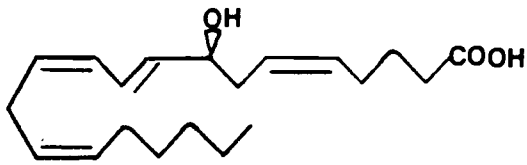


(20) LTD_4[15]
5(S)-hydroxy-6(R)-cysteinylglycyl-7(E),9(E),11(Z),14(Z)-icosatetraenoic acid

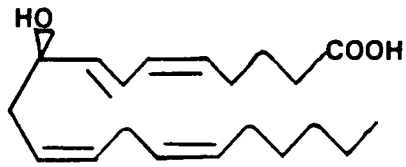
Figure I.1 continued



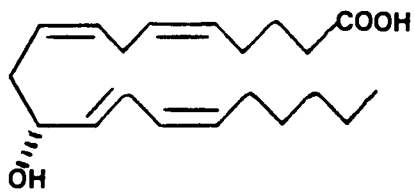
(21) 5-HETE[15]
5(S)-hydroxy-6(E),8(Z),
11(Z),14(Z)-icosatetra-
enoic acid



(22) 8-HETE[15]
8(S)-hydroxy-5(Z),9(E),
11(Z),14(Z)-icosatetra-
enoic acid



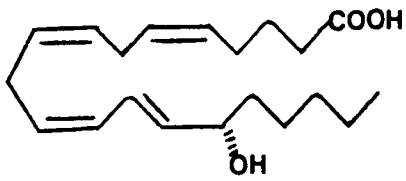
(23) 9-HETE[15]
9(S)-hydroxy-5(Z),7(E),
11(Z),14(Z)-icosatetra-
enoic acid



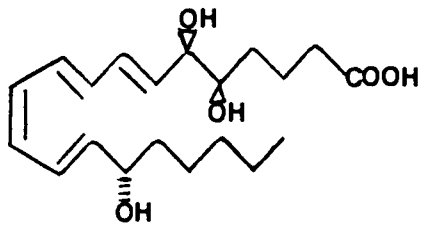
(24) 11-HETE[15]
11(S)-hydroxy-5(Z),8(Z),
12(E),14(Z)-icosatetra-
enoic acid

(25) 12-HETE[15]
12(S)-hydroxy-5(Z),8(Z),
10(E),14(Z)-icosatetra-
enoic acid

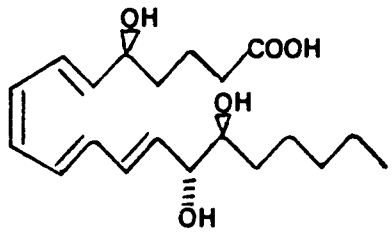
Figure I.1 continued



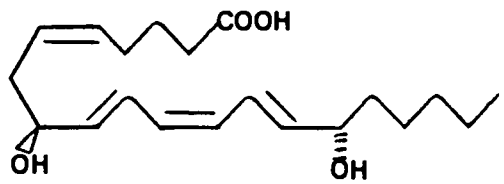
(26) 15-HETE[15]
15(S)-hydroxy-5(Z),8(Z),11(Z),13(E)-icosatetraenoic acid



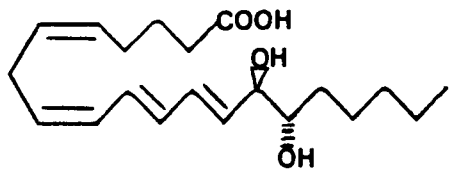
(27) Lipoxin A[42]
5,6,15-trihydroxy-7(E),9(E),11(Z),13(E)-icosatetraenoic acid



(28) Lipoxin B[42]
5,14,15-trihydroxy-6(E),8(Z),10(E),12(E)-icosatetraenoic acid

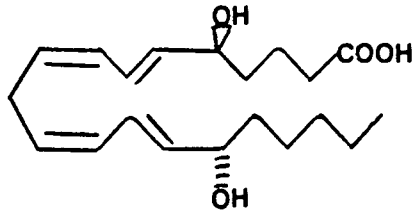


(29) 8,15-DHETE[42]
8(S),15(S)-dihydroxy-5(Z),9(E),11(E),13(E)-icosatetraenoic acid



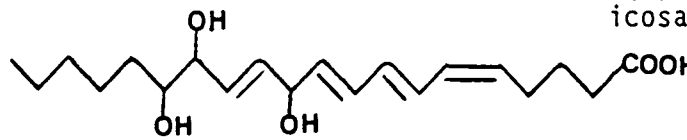
(30) 14,15-DHETE[42]
14(R),15(S)-dihydroxy-5(Z),8(Z),10(E),12(E)-icosatetraenoic acid

Figure I.1 continued



(31) 5,15-DHETE[42]
5(S),15(S)-dihydroxy-
6(E),8(Z),11(Z),13(E)-
icosatetraenoic acid

(32) 11,14,15-THETE[30]
11,14,15-trihydroxy-
5(Z),7(E),9(E),12(E)-
icosatetraenoic acid



(33) 11,12,15-THETE[30]
11,12,15-trihydroxy-
5(Z),7(E),9(E),13(E)-
icosatetraenoic acid

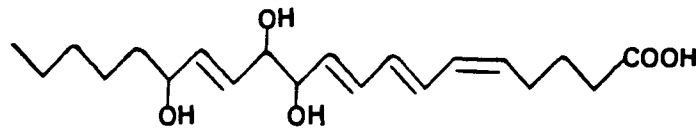
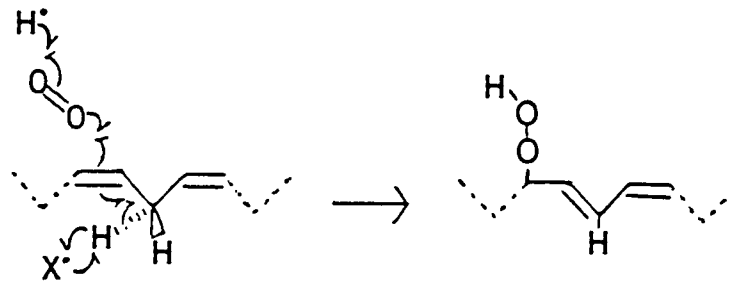


Figure I.1 continued



1. X removes H from 1,4-pentadiene substrate
2. Add molecular O_2 to form a cis, trans diene hydroperoxy-fatty acid

Figure I.2 Lipoxygenase catalysis to form fatty acid hydroperoxide

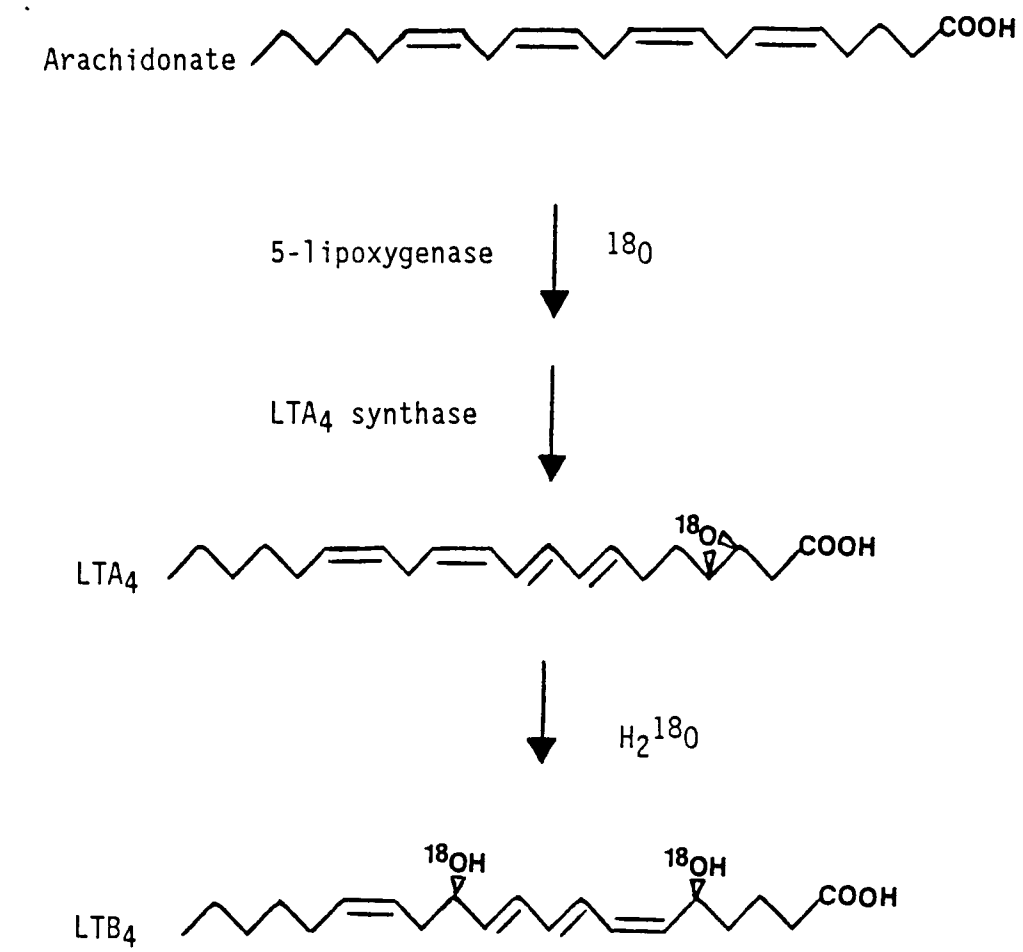
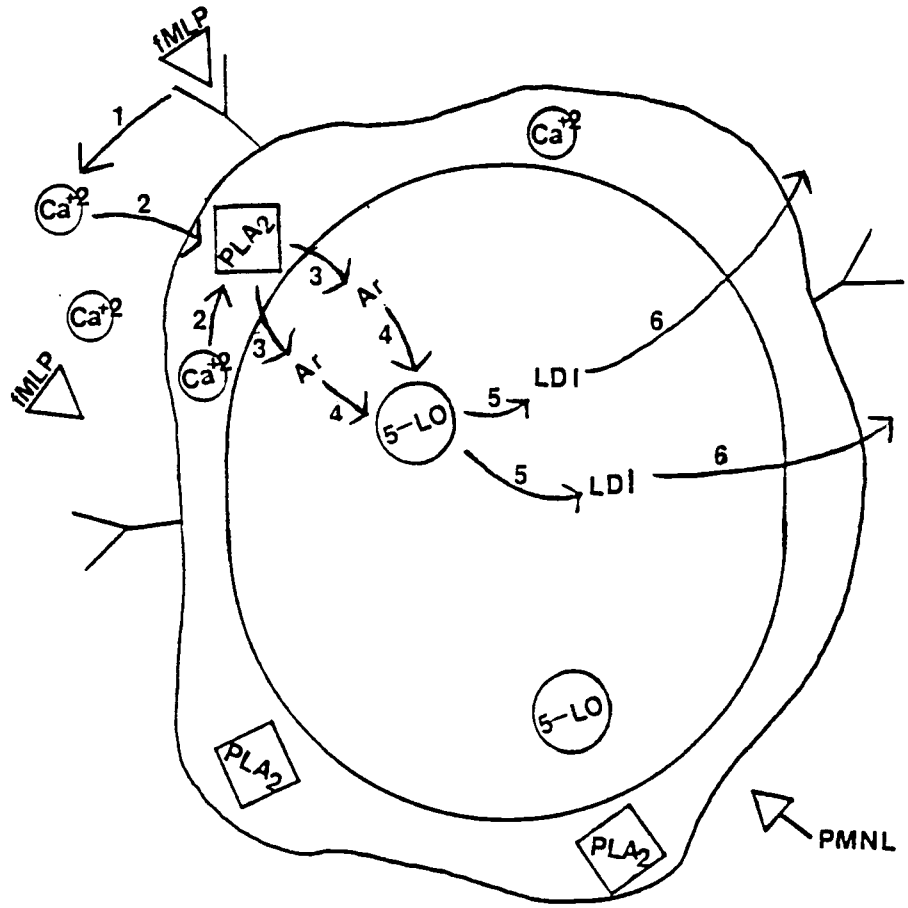


Figure I.3. Biosynthetic pathway of LTB_4



PLA_2 - phospholipase A_2
Ar - arachidonate
5-LO - 5-lipoxygenase
LDI - lipoxygenase-derived icosanoid

1. binding of fMLP causes influx of Ca^{+2}
2. Ca^{+2} activates PLA_2
3. PLA_2 releases Ar from phospholipid
4. Ar substrate of 5-LO
5. 5-LO produces LDI
6. LDI released from cell acts as intercellular mediator

Figure I.4. Role of Ca^{+2} in formation of lipoxygenase-derived icosanoids in leukocytes

Chapter II. Oregon Seaweed Survey

Introduction to the Oregon Seaweed Survey

The need for new and potent drugs has inspired the study of marine organisms as potential sources of biomedicinals.[13] To date, most surveys of marine organisms have been made in temperate and tropical regions,[91,92] and unfortunately, very few studies have been reported for the bioactive constituents of marine organisms from cold water climates.[93] It is important that these organisms not be excluded in the search for useful pharmaceuticals. There is a general hypothesis that cold water algae are less likely to contain biologically active secondary metabolites than tropical algae because of perceived differences in herbivore pressure.[94] Results from the Oregon seaweed survey, which began in 1984 and is currently in progress, fail to support this assumption.

Over 300 different species of algae exist along the Oregon coast.[95] We have collected and surveyed approximately 107 different species of seaweeds since 1984. The cumulative results of this survey are included in Table II.2. The criteria used to screen the various species of algae are as follows: 1. the chromatographic behaviors of the organic metabolites by thin layer chromatography, 2. the antimicrobial activities of these metabolites against 5 types of microorganisms pathogenic to humans, 3. the toxicities of these metabolites towards either gold fish (during 1985) or brine shrimp (from 1986-current). Of

the 107 different species surveyed, over 60% showed some bioactivity, and 51 different species were judged to possess interesting metabolites.

Method of Specimen Collection

The marine algal specimens were collected from rocky regions along the Oregon coastline in the early morning hours of various low tides (usually between -3 and -1). The collections were made between April and October, and most were during the summer months when the algae are more abundant. Specimens were collected in plastic bags and immediately frozen in dry ice. Approximately 100-500 grams dry weight was collected for each specimen. The samples were placed in a freezer (-20°C) until the time of extraction. Frozen vouchers of each specimen were subsequently identified by Dr. William H. Gerwick (Professor of Pharmacy, O.S.U.) and Dr. Harry Phinney (Professor Emeritus of Botany, O.S.U.). Collection sites included Cape Perpetua, Seal Rock, Otter Rock, Yaquina Lighthouse, Marine Gardens and several other points along the southern and northern coastline (Table II.1).

Method of Specimen Extraction

Lipid extracts were produced in a standard fashion[96,97] (Figure II.1). The frozen algae were thawed at room temperature and immediately immersed in solvent ($CHCl_3$/MeOH 2:1, v/v) and

homogenized in a Waring blender until thoroughly chopped. The homogenate was then filtered through cheese cloth, and the filtered algal residue was subsequently suspended in ($MeOH/H_2O$ 7:3, v/v), refluxed for 20 minutes and then filtered and reduced *in vacuo*. The aqueous extract was combined with the aqueous partition fraction from the original filtrate, and was stored in a 25ml glass vial at -5°C. After partitioning of the original filtrate, the organic fraction was reduced *in vacuo*, and the water was azeotropically removed with benzene. The organic fraction was also stored in 25 ml glass vials at -5°C.

Thin Layer Chromatography Analysis

The organic fraction of each specimen was examined for potential novel secondary metabolites using thin layer chromatography (silica gel 60 F254 Merck). The plates were developed using a 1:1 mixture of ether/benzene. Approximately 20 ul of each organic fraction dissolved in diethyl ether was applied to the plate, and the plate was developed for approximately 30 minutes until the solvent front reached within 3.0 cm of the top of the plate. The developed plate was dried and observed under a UV lamp at 254 nm. Any UV active spots were circled, and the previously visible bands were also marked with parentheses. The plate was then lightly sprayed with 50% sulfuric acid and heated on a hot plate. Any charring activity was noted with a check and an abbreviation of its charring color placed next to the spot.

This technique allows the examiner to distinguish between many different types of compounds. Pigments are usually visible and UV active, and may change their color upon charring. Steroids often turn a cherry red initially upon charring. Fatty acids and triglycerides also have distinct UV activities, and upon charring usually turn a grey or brown color. Also, the Rfs (the distance a compound migrates from the origin divided by the distance the solvent migrates from the origin) are characteristic of the type of compound. In normal phase TLC, nonpolar compounds like some pigments and steroids migrate faster than more polar compounds like fatty acids and alcohols. Using the solvent system 1:1 ether/benzene, triglycerides commonly have Rfs between 0.60 and 0.70; Rfs for free hydroxy-fatty acids are usually between 0.10 and 0.30.

In the survey only the organic fraction of each specimen of alga was chromatographed. Both the aqueous and organic fractions were analyzed for their antimicrobial activites and brine shrimp or goldfish toxicities.

Antimicrobial Bioassay

Bioassays (Figure II.2) were used to determine the antimicrobial activities of the aqueous and organic fractions of each seaweed extract. The bioassays done in 1984-1985 were not quantitative, while those assays done during and after 1986 were quantitative. The concentrations of the samples used were 2 mg

for each organic fraction, and 5 mg for each aqueous fraction. Both extracts were tested against five different types of pathogenic microorganisms. The organic extracts were applied to 6.5 mm sterile disks (Difco lab. Detroit, Mi.) using 20 ul of ether as the solvent. The aqueous extracts were applied using 25 ul distilled H_2O as the solvent. Blanks were used for each solvent. The disks were allowed to dry for approximately 30 minutes, and then were placed on agar plates (Mueller Hinton agar, Difco lab.) that had been previously seeded with one of the following microorganisms: Staphylococcus aureus, ATCC 12600, LOT 0385, Candida albicans, ATCC 14053, LOT 0384, Bacillus subtilis, ATCC 6051, LOT 12845, Escherichia coli, ATCC 11775, LOT 06855, Pseudomonas aeruginosa, ATCC 9721, LOT 883. The plates were then incubated overnight at 37°C and the zones of inhibition of growth for each microorganism were measured after a 24 hour incubation period. Only the 1987 survey used ATCC certified microorganisms. For the 1984-1986 survey, microorganisms were provided from stock cultures by the Department of Microbiology, O.S.U..

Brine Shrimp Bioassay

The brine shrimp toxicity assay was used to analyze levels of toxicity at three different concentrations of each organic and aqueous sample. The assay has been compared previously with the cytotoxicities of two human cancer cell lines using over 40 extracts from various Euphorbiaceae species.[98] Over 75% of the

algal extracts that were tested displayed toxicity to brine shrimp with LC50 values less than 1 mg/ml. Brine shrimp eggs were incubated in sea water at room temperature for 48 hours before the assay to allow the eggs to hatch and mature. Approximately 90% of the eggs usually hatch after this incubation period.[98] A shallow rectangular dish filled with artificial seawater (Instant Ocean, Aquarion Systems, Inc.) containing a plastic divider with several 2 mm holes was used to separate the unhatched eggs from the nauphali. The eggs were sprinkled into one compartment that was kept in the dark for the incubation period. After 48 hours, the phototropic nauphlii were collected from the lighted compartment. Approximately 10 shrimp were added to each vial containing either 5 mg, 1 mg or 0.25 mg of aqueous or organic extract in 4.5 ml artificial seawater. Aqueous extracts were introduced to the vials using 50 ul of salt water and 50 ul of 95% ethanol. Organic extacts were introduced using 50 ul of 95% ethanol. Appropriate solvent controls were done in duplicate. The brine shrimp were added to each vial using a long stem pipette. After 24 hours at 20°C the shrimp were counted using a dissecting light microscope, and the percentage of deaths relative to the total number of shrimp was recorded.

Goldfish Toxicity Assay

During 1985, the goldfish toxicity assay was used instead of the brine shrimp assay. The assay involved the introduction of

crude organic or aqueous extracts using either 40 ul MeOH or distilled H_2O as solvent. The extracts were added to 40 ml distilled H_2O in a 50 ml beaker containing a single goldfish, Carassius auratus. The fish was observed by the examiner for one hour, and any behavioral changes relative to the control fish was recorded. Fish unable to maintain a static position when the beaker was swirled were described as experiencing narcosis.

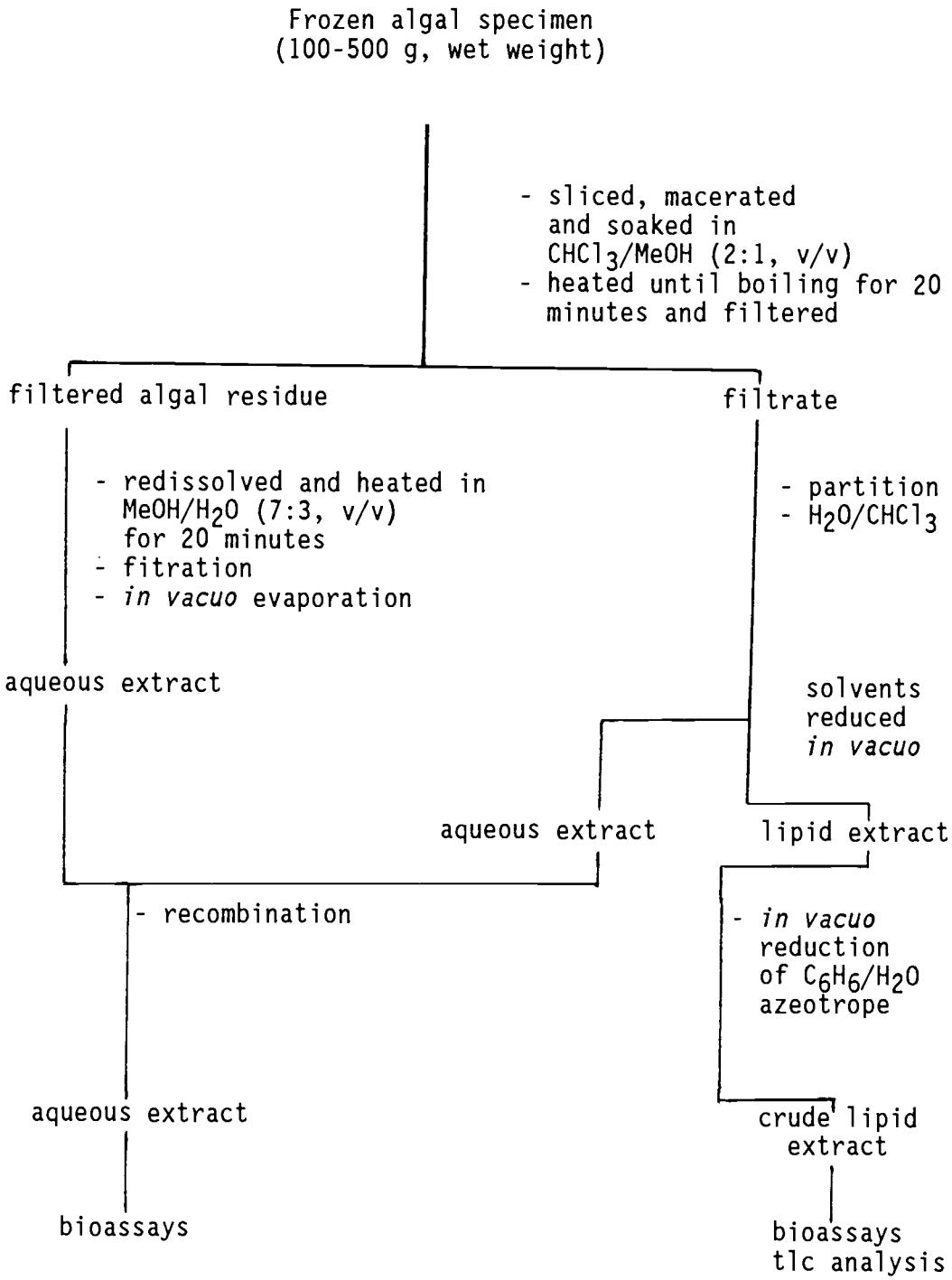
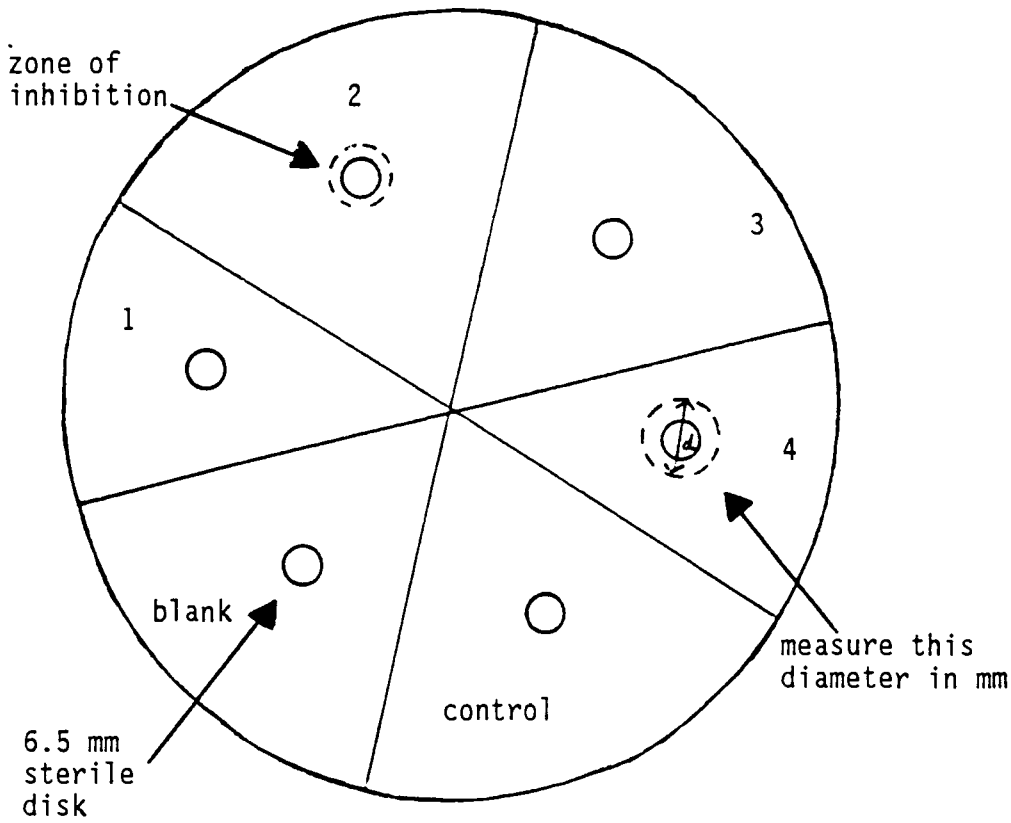


Figure II.1 Generalized extraction scheme.



1-4 = different algal extracts tested at 2 mg/disk for organic extract, 5 mg/disk for aqueous extract

Control + 5 μg streptomycin

Blank = 20 μl ether for organic extract
25 μl dH_2O for aqueous extract

Figure II.2 Diagram of the Antimicrobial Bioassay

Kingdom: Plant

Phylum: Rhodophyta

Class: Florideophyceae

Order: Cryptonemiales

Family: Dumontiaceae

Genus: *Farlowia*

Species: *mollis*

Figure II.3. Taxonomy of *Farlowia mollis* (Harv. & Bail.)

Table II.1

Method for Evaluating the Survey Results

In the tables that follow data will be given on the biological activites and chromatographic behaviors of many of the seaweed extracts that have been evaluated in the Oregon Seaweed Survey.

1. Genus and species names are given along with other information including the names of contaminating organisms and the intertidal collecting region.
2. The code number indicates the area and date of collection along with a recording number: CP = Cape Perpetua, SR = Seal Rock, SH = Strawberry Hill, YH = Yaquina Head, NP = Newport, MG = Marine Gardens, CA = Cape Arego, HH = Heceta Head
3. The zones of inhibition are given for each species of microorganism. The names of these organisms can be found in the description of the antimicrobial bioassay in this chapter. Diameters of inhibition are measured in millimeters. Measurements for the organic sample are given first, followed by the aqueous sample measurements.
4. Observations on the goldfish toxicity assay are given. Abbreviations are explained at the end of Table II.2
5. Unusual spots were recorded by their Rfs on TLC. Brief descriptions of the charring and UV activities were also recorded. The number of +'s indicates the degree and number of interesting metabolites of each species of seaweed.

TABLE II.2 **Oregon Seaweed Survey**
(1984-1987)

PHYLUM Order Genus species	code #	Sa	Bs	Ec	Pa	Ca	Fish	TLC	Shrimp
								Table 4	
CHLOROPHYTA									
Cladophorales									
Chaetomorpha linum (?)	CP-23/7/86-12								
	organic	-	-	-	-	-	ut	(-) UV at .03	
	aqueous	-	-	-	-	-	ut	Char at .4 and .85,	
Chaetomorpha linum	*CP-18/4/87-6	sl	7	-	-	-	ut	(++)ST char .39 UV .25	
		untested					ut	cpds at .05,.06,.18	
								char .54	
Cladophora columbiana	CP-23/7/86-15	8.5	10	10	-	7	ut	(+++) UV at .05,.22	
		-	-	-	-	-	ut	Char at .26,.37,.45	
Cladophora columbiana	*CP-19/8/86-9	untested					ut	(++)ST char .40	100,88,0
							ut	UV .36, char .13,.17,.56	75,13,0
Cladophora columbiana	*YH-18/9/86-3	sl	-	-	-	-	ut	(++) char .39,.54	
		-	-	-	7.5	growth	ut	UV .29	

Table II.2 (continued)

Cladophora columbiana	*SR-16/10/86-7	7.5	sl	-	-	-	ut	(++++)ST char .43 UV .07,	
		-	-	-	11	-			
							ut	.39,.51 cpds at .12,.15,.19,	
								.28,.61	
Cladophora columbiana w/Endocladia muricata	*SH-16/5/87-1	untested					ut	(+++)ST char .37 UV .30,.62	100,0
							ut	char .18,.49 cpds at .10,.33	
Cladophora microcladioides	*CP-18/4/87-2	8	8	-	-	-	ut	(++)ST char .42 UV .17,.30,	
		-	-	-	8	-	ut	.59 cpds at .05,.12	
Cladophora sericea	*CP-19/8/86-7	untested					ut	(+++) char .16,.41st	100,24,0
							ut	UV .37	100,0
Cladophora sp.	CP-23/7/86-11	13	12	-	-	-	ut	(++) UV at .03	
		-	-	-	-	-	ut	Char at .24,.14,.41,.55,.85	
Cladophora sp.	NP-20/6/85-7	9	11	-	-	8	wk	(+) two non-UV active	
		-	-	-	-	-	uk	charring components at .45 & .4	
Cladophora sp.	YH-26/4/86-1	8	11	-	sl	-	ut	(++) UV chromophore at .4	100.22,0
		-	-	-	-	-	ut	Char at .15, .45	38,30,0
Cladophora sp.	CP-23/7/86-27	10	10	-	-	10	ut	(+++) UV at .04,.23,.33	
		-	-	-	-	-	ut	Char at .13,.23,.37,.46	
Spongomorpha coalita	NP-4/6/85-11	-	-	-	-	-	ok	(+++) char only .5,.35,.1	
		-	-	-	-	-	ok	UV active only .2	
								Char and UV at .05,.25	

Table II.2 (continued)

Spongomorpha coalita	SR-26/5/86-25	7.5	8.5	-	-	-	ut	(+++) UV .12,.18,.35 strong	
		-	7.0	-	-	-	ut	UV at .5 weaker	
								Char at .2,.3,.35,.5,.66	
Spongomorpha coalita low intertidal	*MG-6/8/86-1							(+++) char at .37,.53	
								UV & char at .20,.23	
Spongomorpha coalita high intertidal	*MG-6/8/86-2	9	8	-	-	sl		(+++) char at .37,.53	100,89,0
		-	-	-	-	-		UV & char at .20,.23	100,100,87
Spongomorpha coalita	*YH-18/9/86-2	-	-	-	-	-	ut	(++) char .25,.38	
		-	-	-	-	growth			
							ut	UV none	
Spongomorpha coalita	*CA-14/6/87-6						ut	(++++)ST char .36 UV .08,.18,	
							ut	.24,.40,.49 cpds at .22,.29,.44	
								.52	
Spongomorpha saxatilis	MG-22/6/86-8	10	11	7(10)	sl	7.5	ut(+)	3 UV .22,.35,.39 highest	100,0
								running also chars under heat	22,0
		-	-	-	-	-	ut	Char at .15,.20	
Spongomorpha saxatilis	*SR-16/10/86-1	7	sl	-	sl	-	ut	(++)ST char .39, UV .38	
		-	-	-	-	-	ut	char .26,.34,.54 cpd at .19,	
								.22,.56	
Urospora sp. high intertidal	*SH-12/6/87-5	untested					ut	(++)ST char .35 no UV	
							ut	cpds .01,.04,.07,.15,.21	

Table II.2 (continued)

Codiales									
Bryopsis corticulans	NP-20/6/85-14	-	-	-	-	-	ok(++)	three char non-UV active	
		-	-	-	-	-	ok	cpd at .45,.35,.15	
								two uv active cpd at .3,.10	
Codium setchellii	NP-20/6/85-20	7.5	15	-	-	7.5	ok	(+) UV active band at and	
		-	-	-	-	-	ok	slightly above origin	
Codium fragile	CP-23/7/86-13	7	9	9.5	6.5	-	ut	(+) UV .25,.3	
		-	-	-	-	-	ut	Char .4,.47	
Codium fragile	*CP-19/8/86-1	untested					ut	(++) char .41	
							ut	UV none	
Ulotrichales									
Enteromorpha intestinalis	NP-21/6/85-3	-	- halo	-	-	-	ok	(-) sterol at .5 and fat at .6	21.0weak---ok
Enteromorpha intestinalis	*CP-18/4/87-7						ut	(++)ST char .39 UV .26	
							ut	cpds at .07,.12,.53	
Enteromorpha sp.	CP-23/7/86-25	-	8	-	-	-	ut	(++) non-UV and charring at	
		-	-	-	-	-	ut	0.2,.2,.25,.35,.45,.75	
								UV only at 0.05	
Ulva taeniata	SR-26/5/86-9	9.0	9.5	-	sl	7.5	ut	(+) UV .13,.30	100,0
		18.0	sl	sl	sl	?	ut	Char .55,.05	0

Table II.2 (continued)

PHAEOPHYTA									
Chordariales									
Haplogloia andersonii	MG-22/6/86-7	9	11	8	sl	6.5	ut	(-) 1 colored band .19	9,0
								UV active band .27	50,0
Analipus japonicus	NP-21/6/85-5	-	-	-	-	-	ok	(-) charred pigments, fats,	
		10.0	8.5	-	-	-	ok	sterol at usual Rf's	
Leathesia difformis	CP-23/7/86-14	7	9	9	-	-	ut	(++) Char .4, .47	
		-	-	-	-	-	ut	UV .1,.25,.3	
Leathesia difformis	*SH-12/6/87-2	8	-	-	-		ut	(++)ST char .40 LUV .54	100,0
		-	-	-	-		ut	cpd at .03 faint char .21,.24	100,0
Desmarestiales									
Desmarestia ligulata	SR-26/5/86-21	7.5	10	-	-	-	nt	(-) colored char .12	0
		10	12	7.5	10	-	nt	weak UV .30, aqueous extract	0,8,0
								acidic (pH=1) by pH paper	
Desmarestia sp.	YH-11/7/87-1	sl	sl	-	-	-	nt	(-) fats, sterols, faint low	
		-	8.5	-	7.5	-	nt	Rf charring spots	
Dictyosiphonales									
Phaeostrophion irregulare	NP-20/6/85-4	-	-	-	-	-	wk	(-) brown-colored band barely	100,19,0
		13	10	8.0	8.0	-	wk	moving from origin	100,0
Phaestrophion irregulare	SR-26/5/86-16	-	-	-	-	-	ut	(-) 3 bands .05,.09,.15	0
		8	-	-	-	-	ut	.15 chars grey	0

Table II.2 (continued)

Phaestrophion irregulare	SR-26/5/86-18	sl	-	-	-	-	ut	(-) 3 bands .05, .09, .15	0
		-	-	-	-	-	ut	.15 very faint char	0
Soranthera ulvoidea	NP-20/6/85-16	8	8	-	-	-	ok	(++++) 2 char cmpds near	
		-	-	-	-	-	ut	origin brown char cmpds .30,.07	
Fucales									
Cystoseira osmundacea (top portion)	*CA-13/6/87-1	-	-	-	-	-	ut	(++)ST char .36 char .13,	
		7.5	sl	sl	sl	-	ut	.46 UV .22	
Cystoseira osmundacea (base portions)	*CA-14/6/87-10	sl	-	-	-		ut	(+++)ST char .34 no UV	50,33,0
		9.5	8	-	sl		ut	char .07,.48 cpd at .18	66,29,0
Fucus distichus	CP-23/7/86-34	-	sl	11	-	-	ut	(-) 1 char at .36	
		9.5	sl	-	-	-	ut	faint colored band .10	
Fucus sp.	*CA-13/6/87-5	-	-	-	-	-	ut	(+)ST char .34 no UV	
		9.5	-	-	8	-	ut	chars .12,.15,.41,.	
Pelvetiopsis limitata	CP-23/7/86-8	-	-	7	-	-	ut	(+) non UV active chars	29,ut,ut
		-	-	-	-		ut	(+) .5,.45,.6,.8	0
Laminariales									
Alaria marginata	SR-26/5/86-8	-	-	-	-	-	ut	(-) sterol at .5, char at	100,21,0
		8.5	-	-	-	-	ut	.4,.2	15,6,0
Alaria marginata	NP-19/6/85-4	-	-	-	-	-	-	(-) 1 faint band-colored .12	
		9.5	-	-	-	-	-		

Table II.2 (continued)

Alaria marginata (sporophylls)	NP-20/6/85-3	-	-	-	-	-	wk nark	(-) very faint,	
		11	-	-	-	-	wk nark	not interesting	
Costaria costata	MG-22/6/86-14	-	8	-	-	-	ut	(+++) Char .74, UV 0.61,0.50	88,33,0
		-	-	-	-	-	ut	steroid, UV at 0.44, 0.32	75,9,0
Egregia menziesii	*SH-16/5/87-5	untested					ut	(++)ST char .34 UV .23,.27,	
							ut	.48 char .14,.59	
Egregia menziesii	*CA-14/6/87-2	untested					ut		
							ut		
Hedophyllum sessile	MG-22/6/86-16	10	9	-	-	-	ut	(-) fat char 0.58, steroid	16,0
		13	-	-	-	sl	ut	0.48, UV 0.63	80,0
Hedophyllum sessile	CP-23/7/86-26	-	11	-	8	-	ut	(++) Char .14,.24,.38,.46	
		-	-	-	-	-	ut	UV .05,.10,.30	
Laminaria setchellii?	*CA-14/6/87-4	untested					ut	(+)ST char .38 UV .02,.24,.30	
							ut	char .15,.19,.51	
Laminaria sinclairii	SR-26/5/86-3	15	20	17	8	11	ut	(++++) 2 char .05, .2	92,0
		-	-	-	-	-	ut	UV .15,.30,.35	85,78,8
Laminaria sinclairii	*MG-6/8/86-6	untested					ut	(++++) char .50	
							ut	UV .07,.15,.23,.37	
Laminaria sinclairii + Acrochaetium	*SH-16/5/87-6	untested					ut	(+++)ST char .34 UV .19,.25,	100,100,0
							ut	.30,.50,.63 char .09,.14 cpds at.54	80,0
Laminaria sinclairii	*SH-12/6/87-1	untested					ut	(++++)ST char ? UV .19,.30,	
							ut	.38,.43,.54 cpds .08,.13	

Table II.2 (continued)

Laminaria sp.	*SH-16/5/87-4	untested					ut	(++)ST char .34 UV .26,.48	95,100,0
							ut	char .13	0
Macrocystis sp.	*CA-13/6/87-4	8.5	-	sl	12	8	ut	(++)ST char .41 UV .03,.19,	
		-	-	-	-	-	ut	.27 char .14,.52	
Nereocystis sp.	*CA-13/6/87-7	12	11	-	-	8	ut	(++)ST char .34 UV .03,.21,	100,100,18
(blades)		-	-	-	-	sl	ut	.26 char .12,.18,.	83,45,29
Postelsia palmaeformis	NP-21/6/85-1	-	-	-	-	-	ok	(++) 3 UV bands at .14,.20,.25	
(drift)		-	9.5	-	-	8.5	ok		
Postelsia palmaeformis	CP-23/7/86-33	-	6.5	-	-	-	ut	(+) Char .02,.11,.25,.36,.47,	
		-	-	-	-	-	ut	.72, UV .38	
Pterygophora californica	CP-23/7/86-32	-	8	-	-	-	ut	(++) Char .36,.48,.74,.79	
		-	-	-	-	-	ut	UV .23,.27	
Pterygophra sp.	*CA-13/6/87-2	9	-	-	12	sl	ut	(+++)ST char .34 UV .05,.20,	
(blades)		-	-	-	-	-	ut	.27,.47 char .17,.59	
Scytosiphonales									
Scytosiphon lomentaria	MG-22/6/86-18	sl	sl	-	-	-	ut	(+) sterol 0.43	0
		8.0	-	-	-	-	ut	UV 0.35,0.26	23,0
RHODOPHYTA									
Bangiales									
Porphyra lanceolata	SR-26/5/86-24	10.0	11.0	-	-	7.0	ut	(++) Char .10,.15 (.15 also	0
		-	sl	-	-	-	ut	UV), Colored bands .03,.05	0

Table II.2 (continued)

Porphyra nereocystis	*CA-14/6/87-15	untested					ut	(++)ST char .33 UV .16 char	70,37,12
							ut	.35,.48 cpds at .07,.55	
Porphyra sp.	NP-19/6/85-3	10	8.5	-	-	-	narked	(++) 3 UV bands .17,.22,.26	
		-	-	-	-	-	ok		
Porphyrella sp. (?)	NP-21/6/85-6	-	-	-	-	-	ok	(-) fats, sterols and pigments	
		-	-	-	-	-	wk nrk	charred at usual Rf	
Porphyrella gardneri	NP-20/6/85-15	8	10	-	-	-	ok	(-) two char (non-UV) .45,.55	
(on Laminaria)		-	-	-	-	-	ok		
Porphyrella gardneri	*SH-16/5/87-10	8	9.5	-	-	-	ut	(-) only 1 UV at .69	100,100,48
		-	-	-	-	-	ut		all 100
Smithora naiadum	NP-20/6/85-11	-	7.5	-	-	-	ok	(++) Char .55,.25,.23,.20	
		-	-	-	-	-	ok	UV .4	
Ceramiales									
Botryoglossum farlowianum	MG-22/6/86-17	sl	7	-	-	-	ut	(+++) Char & UV 0.53, steroid	14,0
		-	-	-	-	-	ut	0.45, UV 0.74,.0.58,.34,.26	0
Botryoglossum farlowianum	NP-4/6/85-13	-	9	-	-	-	ok	(-) Char 0.45	
		-	-	-	-	-	ok	UV 0.5	
Callithamnion sp.	CP-23/7/86-7	-	10	-	-	-	ut	(+) Char 0.12,0.3	25,ut,ut
		-	-	-	-	-	ut	UV 0.47,0.35	22,0

Table II.2 (continued)

Callithamnion pikeanum	NP-4/6/85-7	-	-	-	-	-	ok	(++) Long UV brown char 0.5	
		-	-	-	-	-	ok	non UV char 0.4, .12	
								UV active, non char .1	
Crytopleura lobulifera	SR-26/5/86-19	7	7.5	-	sl	sl	ut	(++) Colored band .15	0
		-	-	-	-	-	ut	UV active .19	0,8,0
Cryptopleura violacea	SR-26/5/86-13	10	12	sl	8	9	ut	(+) UV active .4 and .22	50,0
		-	-	-	-	-	ut		0
Delesseria decipiens	NP-4/6/85-12	-	-	-	-	-	dead	(++) Char at .55,.45,.15,.05	100,100,18
		-	-	-	-	-	narked	UV & Char at .25	100,100,8
								UV active .5, .2	
Hymenena kylinii(?)	SR-26/5/86-12	sl	8	-	-	7.5	ut	(+) Char .45,.55	14,0
		-	sl	-	-	sl	ut	UV .4	0
Hymenena multiloba	NP-17/1/85-2	untested					ut	(+) UV at .2	
							ut		
Hymenena multiloba	*CA-14/6/87-9	untested					ut	(++++)ST char .34 UV .06,.18,	0
							ut	.22,.26,.29,.42 char .48	70,18,0
Hymenena sp.	NP-20/6/85-5	-	-	-	-	-	dead	(++) Char at 0.5,0.45,.0.35,	
		-	-	-	-	-	ok	.75, UV .2,.15	
Hymenena sp.	SR-26/5/86-15	-	sl	-	-	-	ut	(+) too faint	0
		-	-	-	-	-	ut	no UV bands observed	0
Hymmena sp.	SR-26/5/86-17	sl	9	-	-	-	ut	(+++) 3 colored bands lower	0
		-	sl	-	-	-	ut	Rf's, UV .25,.29	82+s,20,0

Table II.2 (continued)

Hymenena sp.	SR-26/5/86-22	sl	sl	-	-	-	ut	(+) Char .05,.18	7,0
		-	sl	-	-	-	ut	orange colored .11	0
Hymenena sp.	MG-22/6/86-6	13	12	8	-	7	ut	(-) faint, difficult to read	0
		-	sl	-	-	-	ut	2 colored bands at .20,.47	25,10,0
Laurencia spectabilis	SR-26/5/86-11	12	13	10.5	11.5	12	ut	(+++) Char .55,.45,.35,.10	0
		10	7.5	9.0	-	sl	ut	UV .4,.25,.15	73,58+s,8
								one yellow char UV active .30	
Laurencia spectabilis	*MG-6/8/86-3	untested					ut	(+++)Char .20,.23	
							ut	UV .60	
Laurencia spectabilis	*CP-18/4/87-8	untested					ut	(+)ST char .38 UV .07,.11,.20,	
							ut	.32, UV Pig at .65 char .26,	
								.49,.57	
Laurencia spectabilis	*CA-14/6/87-1	untested					ut		
							ut		
Microcladia borealis	MG-22/6/86-4	9	9	8	-	sl	ut	(-) TLC very faint	0
		-	8	sl	-	-	ut	UV active .35	0
Microcladia borealis	CP-23/7/86-3	-	sl	-	-	-	ut	(-) primary metabolites only	
		-	10	-	-	-	ut		
Microcladia borealis	*CP-19/8/86-11	untested					ut		
							ut		
Microcladia sp. on old Nereocystis stipe	*CA-14/6/87-11	untested					ut	(+)ST char .34 no UV	40,33,30
								char .49 cpds at .17,.29	92,80,50

Table II.2 (continued)

Neoptilota densa	NP-17/1/85-1	not tested					narked	(+++) UV 0.5,.02	
							ok		
Neorhodomela larix	YH-3/86-1	17	8	10	8		ut	(+++)ST .35, UV .17,.41	
		10	7.5	10	7		ut	.56 char .27	
Odonthalia flocossa	NP-17/1/85-3	not tested					ok	(++++) UV active brown char	
							ok	.25, lanasol, UV .15	
Odonthalia flocossa	NP-17/1/85-4	not tested					ok	(++++) UV active brown .25,	
							ok	lanasol, UV active .15	
Odonthalia flocossa	CP-23/7/86-2	18	18	14	8	21	ut	(++++) lanasol 0.4, (black	
		-	10	-	-	-	ut	char) UV & purple char at 0.15	
Odonthalia flocossa	CP-23/7/86-4	20	22	18	12	22	ut	(++++) lanasol at 0.4 (black)	
		7	13	-	-	-	ut	UV & purple char at 0.15	
Odonthalia lyallii	*MG-18/9/86-2	10	12	-	-	-	ut	(+++) char .20,.39,.50	
		-	-	-	-	growth	ut	UV .14,.31	
Odonthalia oregona	CP-23/7/86-1	16	12	8	12	11.5	ut	(++++) UV & Purple Char at	
		11.5	12	-	-	-	ut	0.3,0.15,.0.05	
Odonthalia washingtoniensis	NP-4/6/85-2	8	12	-	-	-	ok	(++++) 3 dark brown-black char	
		-	-	-	-	-	ok	low Rfs (lanosol analogues?)	
Odonthalia washingtoniensis	MG-22/6/86-12	24	23	20	8	25	ut	(++++) major grey brown char, UV 0.47, major grey brown UV 0.23, minor grey brown char, UV 0.05	100,100,50
		9	9	-	sl	7.5	ut		0

Table II.2 (continued)

Odonthalia washingtoniensis	*MG-6/8/86-5	untested					ut	(++++) Char .13,.37	
							ut	UV .20,.30	
Polysiphonia hendryi	*SH-16/5/87-8	untested					ut	(+++)ST char .45 UV .27 char .02	
							ut	cpds at .22,.31,.76	
Polysiphonia hendryi w/ Endocladia muricata	*HH-13/6/87-3	11	9-10	-	-	9	ut	(+)ST char .34 UV .25	0
		-	-	-	-	-	ut		100,12,0
Polysiphonia pacifica	MG-22/6/86-5	18	10	10	sl	7	ut	(++) 2 colored bands low	0,33,0
		sl	9	7	-	-	ut	.22,.37, TLC faint	0
Polysiphonia sp. (cystocarpic)	NP-4/6/85-4	10	9	-	-	-	wk nk	(++) UV at .20,.15	
		-	9(?)	-	-	-	ok		
Polysiphonia sp.	NP-4/6/85-14	10	8	7.5	-	-	nark	(++) 3 non-UV active char	
Polysiphonia sp.	NP-21/6/85-4	-	-	-	-	-	ok	(+) UV at .25, fat & sterol	
Pterosiphonia californica	CP-23/7/86-24	8.0	8.0	-	-	-	ut	(+++) Char non-UV .72,.45,.33	
		-	-	-	-	-	ut	UV active .25,.15,.05	
Pterosiphonia sp.	NP-20/6/85-10	11(21)	12 (18)	-	7.5(7)	-	dead	(+++) char .45,.4	
		-	-	-	-	-	ok	UV active .25, fat & sterol	
Ptilota filicina	NP-9/6/85-1	9	8	-	-	-	ok	(++++) Major UV brown .25	
		-	15 halo	-	-	-	narked	0.4, minor red char at .2	
Ptilota filicina (w/diatoms)	NP-20/6/85-19	7(19)	7(12)	-	-	-	narked	(+) Char band .10	
		-	7(9)	-	-	-	ok	UV active .14	

Table II.2 (continued)

Ptilota filicina	YLH-28/2/86-1	not tested					ut ut	no TLC	
Ptilota filicina	MG-26/4/86-1	not tested					ut ut	(++++) red char .10, major red .2, UV .6 (brwn), .5 (drk brn, major) .25 (brwn)	
Ptilota filicina	SR-26/5/86-2	not tested					ut ut	(++++) Red char UV active .15 Brown char UV active .5 Char at .6,.75	
Ptilota filicina	MG-22/6/86-1	not tested					ut	(++++) Red char UV active .2 brown char UV active .5 pink char (sterol) .65, brwn char (fat) .75	
Ptilota finicina	MG-4/7/86-1	sl	sl	-	-	-	ut	(+) (5% MeOH/Chl) pink char .75, red char at .05	
Ptilota filicina	MG-6/8/86-4	not tested					ut ut	(+) (5% MeOH/Chl) black char UV active .75 char (pink) .6 (sterols)	
Ptilota filicina	*MG-18/9/86-1	- -	- -	- -	- sl	- growth	ut	(++) char .39,.53 utUV .21	
Ptilota filicina	*SH-16/5/87-2	untested					ut ut	(+)ST char .34 UV .49	
Ptilota filicina	*HH-13/6/87-4	- -	- -	- -	- -	- -	ut ut	(-)St char .34 UV .30, .43 cpd at .21	22,0 0

Table II.2 (continued)

Cryptonemiales									
Bossiella plumosa	MG-22/6/86-2	-	8	7	-	-	nt	(++++) UV active .15,.5	0%
		-	13	-	-	-	nt	UV active .7,.55,.47,.25	0%
Bossiella sp.	CP-23/7/86-10	-	12 halo	-	6.5	-	ut	(+) Char .25,.58,.88	100,0,7[z]
		-	-	-	-	-	ut	UV .4,.83,.87	6,0[z]
Calliarthron sp.	NP-19/6/85-2	-	7(12)	-	-	-	ok	(-) UV active .15	
		-	-	-	-	-	ok		
Callophyllis sp.	NP-4/6/85-8	-	-	-	-	-	ok	(-) UV active non-char 0.4	
		-	8	-	-	-	ok		
Callophyllis sp.	SR-26/5/86-10	-	sl	-	-	-	ut	(-) UV .4	0
		-	sl	-	-	-	ut	Char .45	0
Constantinea simplex	SR-26/5/86-20	-	-	-	-	-	ut	(-) TLC faint - one colored	0
		-	sl	-	-	-	ut	band .10	0
Constantinea simplex	CP-23/7/86-6	-	-	-	-	-	ut	(-) fat char 0.48, sterol 0.4	
		-	-	-	-	-	ut	UV no char at 0.27,.07	
Corallina sp.	CP-23/7/86-22	sl	9.0	-	-	-	ut	(+++) Char UV-non active	
		-	-	-	-	-	ut	.75,.45,.33,.25	
								UV active .15,.37	
"Coralline Red"	MG-22/6/86-3	sl	7.5	8	-	-	ut	(-) UV active .20	0%
		-	-	-	-	-	ut		0
Dilsea californica	SR-26/5/86-6	8	13	-	sl	-	ut	(-) UV active 0.5	0
		-	-	-	-	-	ut		14,11,0

Table II.2 (continued)

Dilsea californica	MG-22/6/86-11	-	-	-	-	sl	ut	(-) UV 0.47	100,67,45
		sl	-	-	-	-	ut		50,0
Endocladia muricata	NP-20/6/85-12	-	-	-	-	-	wk nrk	(+) non UV char .55,.45	
		-	-	-	-	-	wk nrk	UV active char .33	
Endocladia muricata	*CA-14/6/87-14	sl	-	-	-	-	ut	(+)ST char .34 char .21,.48	100,19,0
		-	-	-	-	-	ut	cpds at .02,.07,.29,.42	80,100,8
Farlowia mollis	CP-23/7/86-35	8	11	13	7	9	ut	(++++) blue after acid spray	
		-	8	-	-	-	ut	chars gray-black .13	
								large UV active char .42	
Farlowia mollis	*CP-19/8/86-2	untested					ut	(+++) char .41st,.79	
							ut	LUV .57	
Farlowia mollis	*CP-19/8/86-10	untested					ut	!	
							ut		
Farlowia mollis	*SH-16/5/87-12	-	-	-	-		ut		80+2,71,0
		-	-	-	-		ut		100,40,10
Farlowia mollis high intertidal	*CA-15/6/87-1	untested					ut		
							ut		
Farlowia mollis	*HH-13/6/87-2	-	-	-	-	sl	ut	(+++)ST char .34 UV .31,.42	100,72,50
		-	-	-	-	-	ut	cpd at .14	10,0
Gloiosiphonia capillaris	NP-20/6/85-17	-	-	7.5	-	-	-	(+++) UV active .09,.22	100,100,27
		-	-	-	-	-	-		100,100,91
Gloiosiphonia capillaris	*SH-12/6/87-6	12	9	9	-	11	ut	(++++)ST char .35 UV .06,.17,	80,0
		-	-	-	-	-	ut	.21,.27 char .09,.40,47	63,10,0

Table II.2 (continued)

Gloiosiphonia verticillaris	*SH-16/5/87-15	8	8	7	-	10	ut	(+++)ST char .36 UV .10,.20,	85,100,100
		-	-	-	-	-	ut	.70 char .08,.15,.46 cpds at .02,.03	92,100,11
Halymenia sp.	NP-19/6/85-5	13	9.0	-	-	-	nark	(+++) Char at low Rf's	
		-	-	-	-	-	ok		
Prionitis lanceolata	SR-26/5/86-14	7.5	9.5	-	-	-	ut	(++++) Long UV .5,.55,.6	
		-	10	-	-	-	ut	Char .45	
								UV active (pinkish) .20	
Prionitis lanceolata (tidepool ?)	*CP-19/8/86-3	untested					ut		100,0
							ut		10,0
Prionitis lanceolata (surfform)	*CP-19/8/86-4	untested					ut		
							ut		
Prionitis lanceolata	*YH-18/9/86-1	-	-	-	-	-	ut	(++++) char .37	
		-	-	-	-	growth	ut	UV .17	
								LUV .03,.04,.07,.13,.17 .19,.21,.45,.50,.53	
Prionitis lanceolata	*YH-18/9/86-4	-	-	-	-	-	ut	(++++) char .37	
		-	-	-	-	growth	ut	UV .17,.21	
								LUV .03,.04,.07,.13,.19 .32,.37,.45,.50,.53	
Prionitis lanceolata	*SR-16/10/86-5	sl	sl	-	-	-	ut	(++++)ST char .40 UV .05,	
		-	-	-	-	-	ut	.07,.17,.26,.72 LUV .53	
								char .13,.21,.64	

Table II.2 (continued)

Prionitis lanceolata	*CP-18/4/87-1	untested					ut		
							ut		
Prionitis lanceolata	*SH-16/5/87-14	13	10-11	al	-	9	ut	(++++)ST char .34 UV .06,	100,69,50
		-	-	-	-	-	ut	.15,.21,.27,.40 cpds at .51,.58	100,0
Prionitis lanceolata	*HH-13/6/87-1	-	-	-	-	al	ut	(+++)ST char .32 UV .30,.59	31,10,0
		-	-	-	-	-	ut	char .44 cpds at .07,.14,.21, .47	50,100,0
Prionitis linearis(?)	CP-23/7/86-28	7	7	-	-	-	ut	(-) No UV activity	
		-	7	-	-	-	ut	Char .37,.50,.80	
Prionitis linearis	*CP-19/8/86-5	untested					ut	(+++) char .06,.09,.41at,.56	100,0
							ut	UV .75	100,20
Prionitis linearis	*CP-18/4/87-10	-	-	-	al	-	ut	(++++)ST char .38 UV .21,.24,	
		-	-	-	-	-	ut	.52,.71 char .07, cpds at .12, .18	
Prionitis linearis + Bryozoans	*SH-16/5/87-11	untested					ut	(+++)ST char .34 UV .13,.48,	100,100,12
							ut	.62,.69 cpds at .21,.57	100,100,70
Prionitis linearis	*HH-13/6/87-5	-	-	-	-	-	ut	(+)ST char .33 UV .01	43,8,0
		-	-	-	-	-	ut	cpd at .21	50,50,0
Prionitis lyallii (lanceolata?)	CP-23/7/86-9	9	7	14	-	12	ut	(++++) UV .1,.2,.25,.47	54,ut,48
		-	-	-	-	-	ut	.2 chara heavily (purple) Char .03	36,15,0
Prionitis lyallii? low intertidal	*SH-12/6/87-3	-	-	-	-	al	ut	(++++)ST char .40 UV .05,.08,	100,100,70
		-	-	-	-	al	ut	.24,.28,.52 cpds at .01,.02, .17,.20,.40,.47,.51,.55,.62	85,50,14

Table II.2 (continued)

Prionitis lyallii? high intertidal	*SH-12/6/87-4	untested					ut	(++++)ST char .40 UV .08,..24,	
							ut	.25,.28,.47,.52 LUV .52	
								cpds at .01,.02,.17,.20,.40,	
								.47,.51,.62,.64	
Prionitis sp.	NP-20/6/85-1	-	-	-	-	-	wk nrk	(-) not enough to observe	
		-	-	-	-	-	wk nrk		
Prionitis sp.	CP23/7/86-20	-	-	-	-	-	ut	(++++) Non-UV Char .33	
		-	-	-	-	-	ut	UV active .15,.08,.05	
Prionitis sp.	CP-23/7/86-29	9	9	-	7	8	ut	(++++) UV .07,.16	
		-	12	-	-	-	ut	Char .36,.44	
Prionitis sp. (exposed surf)	CP-23/7/86-36	-	-	-	-	-	ut	(++++) red char .16	
		-	-	-	-	-	ut	UV .07 to origin	
Prionitis sp. (tidepool)	CP-23/7/86-37	7.5	8.5	-	-	-	ut	(++++) red char .16	
		-	7.0	-	-	-	ut	UV .07 to origin	
Prionitis sp.	*CP-19/8/86-8	untested					ut	(++++)ster. char .45	100,11,0
							ut	UV .17,LUV .03,.12,.61	100,56,0
Prionitis sp.	*CA-13/6/87-6	7.5	-	8	8	7.5	ut	(+++)ST char .34 UV .02,.07,	
		-	-	-	-	-	ut	.15,.17,.21,.43 char.47	
Gigartinales									
Ahnfeltia plicata	NP-20/6/85-13	7.5	7.5	-	-	10	ok	(++) Char non-UV .2,.35,.55,	all 100
		-	-	-	-	-	ok	.75, UV active .5	100,100,44
Gigartina papillata	CP-23/7/86-23	8	8.5	10	-	8.5	ut	(-) Char non UV .45,.33	
		-	-	-	-	-	ut		

Table II.2 (continued)

Gigartina papillata	*MG-18/9/86-3	-	sl	-	-	-	ut	(++) char .23,.39,.54	
		-	-	-	sl	-	ut	UV .30,.39,.56	
Gigartina volans	SR-26/5/86-5	-	8	-	-	-	ut	(-) Pigments, fats, sterols	0,0,0
		-	-	-	-	-	ut	only	22,0,0
Gigartina sp.	NP-19/6/85-1	-	-	-	-	-	ok	(+) UV active .15	
		-	-	-	-	-	ok		
Gigartina sp.	CP-23/7/86-21	-	-	-	-	-	ut	(-) One non UV .33	
		sl	sl	-	-	-	ut		
Gigartina sp.	*CA-13/6/87-3	8.5	-	-	10.5	sl	ut	(-)sterols, lipids,	
		-	-	-	-	-	ut	UV at .04	
Gigartina sp.	*CA-14/6/87-7	untested					ut	(-)ST char .36 no UV	25,0
							ut	lipids, + cpds at .05,.11,.18	100,30,10
Gracilaria sjoestedtii	*SR-16/10/86-3	untested					ut		
							ut		
Gymnogongrus (linearis?)	NP-20/6/85-2	-	-	-	-	-	ok	(+) TLC faint, uninteresting	
		-	-	-	-	-	ok		
Gymnogongrus linearis	*SR-16/10/86-2	-	-	-	-	-		ut(++)ST char .40,UV .12,.14,	
(+ G. leptophyllus)		-	-	-	-	-	ut	utchar .16,.36,.55	
Gymnogongrus linearis	*SH-16/5/87-13	-	-	-	-	7	ut	(++)ST char .36 UV .22,.54,	
		-	-	-	-	-	ut	.69 char .43 cpds at .15,.20	
Hypnea sp.??	*CA-14/6/87-5	untested					ut	(+++)ST char .36 UV .06,.33,	
							ut	.57 char .25,.50 cpds at .03,	

Table II.2 (continued)

Iridaea cordata	*CA-14/6/87-12	16	12	10	sl	13	ut	(+++)ST char .33 UV .23,.49,	80,71,0
		-	-	-	-	-	ut	.57,.65 char .52 cpds at .36, .07,.18,.34	all 100
Iridaea heterocarpa	SR-26/5/86-23	8.5	11	-	-	8	ut	(-) Char (gray) 0 up to .13	0
		-	sl	-	-	-	ut	weak UV active .27	0
Iridaea heterocarpa	CP-23/7/86-5	-	-	-	-	-	ut	(-) primary metabolites only	
		-	-	-	-	-	ut		
Iridaea sp.	CP-23/7/86-30	10	10	-	9	8	ut	(++) Char .37,.47,.78	
		-	-	-	-	-	ut	UV .06,.10,.26,.32	
Iridaea sp.	*HH-13/6/87-7	13	8	-	-	7	ut	(++++)ST char .36 UV .30,.50	57,25,9
		-	-	-	-	-	ut	char .04,.08,..09,.12,.16 cpd at .24	0
Plocamium (cartilagineium)?	NP-20/6/85-6	8	9.0	7.5	-	9	dead	(++++) UV .15 to .25	
		-	-	-	-	-	wk nrk	UV char .3,.75 Non-UV active char .4,.35,.65	
Plocamium violaceum	*SH-16/5/87-9	9.5	8.5	sl	-		ut	(++++)ST char .34 UV .22,.28,	100,92,60
		-	-	-	-		ut	.37,.44,.53,.67,.75	100,33,10
Plocamium violaceum	*HH-13/6/87-6	-	-	-	-	-	ut	(++)ST char UV .59	0,7,0
		-	-	-	-	-	ut	cpd at .16,.51 char .43	56,13,0
Rhodoglossum sp.	NP-20/6/85-9	-	-	-	-	-	ok	(-) Char .45,.4	
		-	-	-	-	-	ok	(fat & sterol)	
Schizymenia pacifica	SR-26/5/86-4	-	-	-	-	-	ut	(++) UV active .6 (fat)	0
		-	-	-	-	-	ut	UV active .5, Char .45(sterol)	0

Table II.2 (continued)

Nemaliales									
Cumagloia andersonii	NP-20/6/85-18	-	-	-	-	-	ut	(-) too faint to read	
		-	-	-	-	-	ut	uninteresting	
Cumagloia andersonii	NP-21/6/85-2	-	-	-	-	-	ok	(-) colored band near origin	
(Marine Gardens)		-	-	-	-	-	ok		
Gelidium	SR-26/5/86-7	8	12	-	-	8	ut	(+++) Char at .4,.35,.3,.05	
(pusillum?)		sl	sl	-	-	sl	ut	UV at .3,.15,.1	0
Rhodymeniales									
Halosaccion glandiforme	NP-20/6/85-8	-	-	-	-	-	wk nrk	(-) non-UV active char	
		-	-	-	-	-	ok	.4 (sterol)	
Unidentified Rhodphytes									
Foliose red	CP-23/7/86-19	8.5	9.5	-	-	9	ut	(-) Char .85	
		-	-	-	-	-	ut	UV .47	
Foliose red	*CA-13/6/87-8	14	9	-	-	9	ut	(+++)ST char .34 UV .08,.	63,20,0
		-	-	-	-	-	ut	.13,.20,.25,.50,.63 cpds at .03,.05	11,0
Red (unknown)	*CA-14/6/87-8	untested					ut	(+++)ST char .35 UV .51 char	100,80,0
							ut	.20,.26,.46,.75 cpds at .01-.06,.14	100,0
Red (unknown)	*CA-14/6/87-13	untested					ut	(++)ST char .34 no UV	45,0
							ut	char .48 cpds at .29,.34,.41	100,87,50

Table II.2 (continued)

Small red	CP-23/7/86-31	6.5	8	7	-	7	ut	(-) Char .36,.44,.82	
		-	-	-	-	-	ut	UV .28	
Chrysophycophyta									
Navicula cumoides	NP-4/6/85-3	-	-	-	-	-	ok	(-) UV active .15	
		-	-	-	-	-	ok	UV active, char .5	
Diatoms (on odonthalia	*MG-6/8/86-7	-	-	-	-	-	ut	(++) char .17,.37,46	100,86,27
		-	-	-	-	-	ut	UV .27,.53	100,100,82
Diatoms?	*CA-14/6/87-3	untested					ut		
							ut		
MOTILE ANIMALS									
Jellyfish "sailors"	*CA-13/6/87-9	-	-	-	-	-	ut	(++)ST char .33 char .46,.52	0
		-	-	-	-	-	ut	cpds at .07,.08,.11,.23	100,0
SESSILE ANIMALS									
Bryozoan 1	CP-2/37/86-16	7	7	7	-	-	ut	(-) Chars .11,.37,.48,.81	
		-	-	-	-	-	ut		
Bryozoan 2	CP-23/7/86-17	7	8	-	-	-	ut	(+) Char .07,.38,.48,.52,.80	
		-	-	-	-	-	ut	UV .31,.72,.76	
Bryozoan 3	CP-23/7/86-18	-	7.5	-	-	-	ut	(+) Char .37,.46,.55,.70	
		-	-	-	-	-	ut	UV .16,.3,.64,.74,.78	
Nudibranchs feeding on SH-12/6/87-8	*SH-12/6/87-7	-	-	-	-	-	ut	(+++)ST char .34 UV .10,.34	100,100,91
		-	-	-	-	-	ut	char .27,.30,.37,.48	83,45,29

TableII.2 (continued)

Sponge	*SH-16/5/87-7	untested					ut	(+)ST char .49 UV .32	
w/ zoochlorellae							ut	cpd at .30	
Sponge	*SH-12/6/87-8	-	-	-	-	8	ut	(+++)ST char .34 char .20	
Sponge	*SR-15/7/87-1	6.5	-	-	-	-	ut	(+++) char .35st,.30,.42,.56	
(Halichondria panicea)		-	8	-	7		ut	UV .15,.33	
								10% MeOH in chloroform	
								TLC char .66st,.03,.08,.18,	
								UV base,.18,.23	
								20% MeOH in chloroform	
								TLC char .69st,base,.20,.42,	
								.54 UV base,.15,.22,.32,.42,.54	
"brain sponge"	YH-11/7/87-2	18	20	-	-	13	ut	(+++) many hi & lo UV chars	
Tube Worms	MG-22/6/86-13	7.0	-	-	-	7.0	ut	(+) UV 0.66, 0.70	44,22,0
		sl	-	-	-	growth	ut		0
Unidentified organism	MG-22/6/86-10	7.0	8	-	-	sl	ut	(++) UV & grey char 0.53	75,33,0
		sl	-	-	-	-	ut	UV 0.4	
ANGIOSPERMS									
Phyllospadix	MG-22/6/86-9	14	13	-	-	7	ut	(-) UV 0.23,.0.47	15,0
		-	-	-	-	-	ut		17,0
Phyllospadix scouleri	*SH-16/5/87-3	untested					ut	(++++)ST char .34 UV .18,.30,	
							ut	.35,.42,.51,.57	

Table II.2 (continued)

ATLANTIC SP.								
Polysiphonia	*RB-10/6/87-1	-	-	-	-	-	ut	0
subtilissima(Atlantic)		-	-	-	-	-	ut	0

footnotes

z the aqueous and lipid extracts for the brine shrimp assay may be reversed (see general bioassay book 1, 26 April 1987) for each specimen number, data will be given on two lines. The first line will be for the lipid extract. The second line will be for the aqueous extract

abbreviations for goldfish toxicity assay

- sl - slight inhibition
- ut - untested
- ok - no effect
- wn - weak narcosis
- n - narcosis
- sn - strong narcosis
- d - dead

Table II.3 Summary of the Bioactivity and Chemistry in Different Phyla of Algae from Oregon

Phylum species	gram (+) bacteria	gram (-) bacteria	yeast	brine shrimp	potential new chemistry
Rhodophyta 63	83%(52)	44%(28)	48%(30)	44%(28)	51%(32)
Phaeophyta 26	81%(21)	42%(11)	23%(6)	57%(15)	38%(10)
Chlorophyta 18	67%(12)	50%(9)	38%(7)	33%(6)	50%(9)
107 species	85	48	43	49	51

CHAPTER III. Isolation and Spectroscopic Identification of Novel Icosanoids from the Red Marine Alga *Farlowia mollis*

Abstract

The Oregon red seaweed Farlowia mollis was found to contain three icosanoids possessing novel sites of oxidation and unsaturation as well as two previously described icosanoids. The structures of the new compounds were determined by detailed spectroscopic analyses including the extensive use of ^{1}H-^{1}H COSY performed on stabilized acetate-methyl ester derivatives. Mixtures of the new seaweed compounds show several pharmacological activities typical of mammalian-derived icosanoids, including inhibition of fMLP-induced superoxide anion generation by human neutrophils, inhibition of conversion of arachidonic acid to lipoxygenase products by human PMN leukocytes and inhibition of dog kidney Na^{+}/K^{+} ATPase.

Introduction

Recently, our laboratory reported on the isolation of several new icosanoids from the Oregon red seaweed Ptilota filicina.[12,26] Our continued survey of Oregon seaweeds for new natural products with potential biomedicinal application has now identified another red alga, Farlowia mollis (Harv. & Bail.) Farl. & Setch., as a rich source of structurally novel and physiologically active icosanoids.[100] The structures of these unstable metabolites, farlowdiols A-C (**34-36**), were efficiently solved by application of 2D-NMR methodology on stabilized derivatives. Furthermore, a metabolite recently isolated from P. filicina, 5(Z),7(E),9(E),-14(Z),17(Z)-icosapentaenoic acid (**5**), as well as icosapentaenoic acid, were also present in the organic extract of this cold water seaweed.

The delicately branched red alga Farlowia mollis was originally collected in our survey efforts from Cape Perpetua in July 1986. The lipid extract from this collection showed antimicrobial activity to several human pathogens and the occurence of potentially novel compounds by TLC (blue char upon acidification at 0.13 R_f). Thus, larger collections were made in August 1986, May 1987 and June 1987, all of which contained unusual appearing blue charring compounds. Bioautography of this extract indicated that polar compounds in the extract were responsible for the antimicrobial activity. Hence, the material collected in August

of 1986 was vacuum chromatographed over silica gel to rapidly yield mixtures enriched in these polar blue-charring compounds. In order to stabilize these compounds and render them more easily separable, they were first treated with CH_2N_2 and later with acetic anhydride in pyridine to produce diacetate methyl ester derivatives. This mixture of derivatives was then easily separated employing HPLC to yield a purple charring compound, farlowdiol A diacetate-methyl ester (**37**), and a blue charring compound, farlowdiol B diacetate methyl ester (**38**). A slightly more polar fraction from the original vacuum chromatography yielded, following similar derivations, a related compound, farlowdiol C diacetate methyl ester (**39**).

Experimental Methods

Ultraviolet spectra were recorded on an Aminco DW-2a UV-Vis spectrophotometer, and infrared spectra (IR) were recorded on a Nicolet 5 DXB FT 15 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM 400 NMR spectrometer and all shifts are reported relative to an internal TMS standard. Low resolution mass spectra (LRMS) were obtained on a Varian MAT CH7 spectrometer, while high resolution mass spectra (HRMS) were obtained on a Kratos MS 50 TC. High performance liquid chromatography (HPLC) was done using a Waters M-6000 pump, U6K injector and R 401 differential refractometer, while thin layer chromatograms were made using Merck aluminum-backed TLC sheets

(silica gel 60 F_{254}). All solvents were distilled from glass prior to use.

Farlowia mollis was collected from exposed intertidal pools (-0.5 to +0.5m) at Cape Perpetua on the Oregon coast in August 1986. The seaweed was preserved by freezing until workup, at which time the defrosted alga (103 g dry weight) was homogenized in warm $CHCL_3$/MeOH (2:1,v/v). The mixture was filtered and the solvents were removed in vacuo to yield 600 mg of a dark green tar. The $CHCL_3$ was filtered, reduced in vacuo and the water azeotropically removed with benzene. The crude extract was fractionated by silica gel chromatography in the vacuum mode (10cm x 9cm, Merck TLC-grade Kieselgel), and metabolites were progressively eluted with increasingly polar mixtures of isooctane and ethyl acetate. Those eluting with 50% EtOAc/isooctane yielded a mixture of fatty acids containing compounds **34**,**35** and **36**. Treatment of a portion of these fractions with CH_2N_2 afforded a mixture of methyl esters that was subsequently chromatographed by normal phase HPLC (μ-Porasil Z-module, 65% EtOAc/isooctane) which removed residual pigments from the fatty acid mixture. After observations using NMR, the fatty acid mixture was acetylated using excess acetic anhydride/pyridine (1/1) and the resulting mixture was separated by normal phase HPLC (2 x 3.9 mm x 25 mm μ-Porasil, 10% EtOAc/isooctane) to yield 10mg of **37**, 15mg of **38** and 4mg of **39**.

Methyl 12,13-diacetoxy 5(Z),8(Z),10(E),14(Z)-icosatetraenoic acid **37**. Compound **37** was a colorless oil showing the following: UV (MeOH) λ_{max} 236 nm (ϵ = 34,069); IR ($CHCL_3$) ν_{max}: 3022,3019,2931,1734,1372,1246,1222,1217,1210,1026 cm^{-1}; $(\alpha)_D^{25}$ = +2.24 (c=0.63, CCL_4); Low resolution electron impact mass spectrometry (LR EIMS) m/z (rel. intensity) 374(M^+) - AcOH(9.1), 314(M^+) - 2AcOH(4.4),265(26.1),225(22.0),223(82.7),205(36.7), 191(20.5),173(12.8),169(22.6),163(17.3),131(14.5),127(62.9), 95(11.2),91(14.4),83(15.3),81(23.3),79(16.6),67(20.2),57(20.2), 55(20.6),43(100); HR EIMS m/z obs.374.24768 (M+) - AcOH,$C_{23}H_{35}O_4$ requires 374.24757; For ^1H NMR and ^{13}C NMR data see Table III.1.

Methyl 12,13-diacetoxy 5(Z),8(Z),10(E),14(Z),17(Z)-icosapentaenoic acid **38**. Compound **38** was also isolated as a colorless oil and showed UV (MeOH) λ_{max} 236 nm (ϵ = 27,116); IR ($CHCL_3$) ν_{max}: 3020,2965,2955,1734,1434,1372,1223,1220, 1208,1025 cm^{-1}; $(\alpha)_D^{25}$ = +3.72 (c=1.13,CCL_4); LR EIMS m/z (rel. intensity) 372(M+) - AcOH(6.6),312(M^+) - 2AcOH(4.7),265(29.6), 237(17.0),224(15.5),223(100),205(48.8),191(28.0),173(21.0),163 (28.6),147(13.7),145(20.2),141(16.6),135(10.1),131(28.9),129 (11.9),125(32.5),121(13.2),109(14.7),107(93.0),105(19.0),97(10.1), 95(24.0),93(20.3),91(36.3),83(22.8),81(35.0),80(12.5),79(53.2), 77(13.2),71(16.3),69(15.6),59(13.1),57(20.3),55(37.1); For ^1H NMR and ^{13}C NMR data see Table III.1.

Methyl 10,11-diacetoxy 6(Z),8(E),12(Z)-octadecaenoic acid **39**. Compound **39** was also isolated as a colorless oil showing the following: UV (MeOH) λ_{max} 234 nm, (ϵ = 16,755); IR (neat) ν_{max} 3014,2954,2930,2858,1742,1457,1437,1371,1241,1226,1168,1114, 1104,1026 cm^{-1}; LR EIMS m/z (rel. intensity) 348(M^+) - AcOH(13.5), 239(25.4),237(10.7),223(15.9),198(14.6),197(73.5),180(12.5), 179(100),169(24.4),165(62.4),147(25.0),137(10.8),127(63.1),119 (19.4), 109(21.1),107(13.7),105(11.7),95(10.8),93(10.6),91(14.6), 83(10.8),81(22.4),79(14.3),67(18.9),57(19.4),55(16.9),43(78.9); For ^1H NMR and ^{13}C NMR data see Table III.1.

Preparation of the Bromobenzoate Derivatives for the Assignment of Absolute Stereochemistry

Approximately 400 mg of Farlowia mollis crude organic extract was methylated using CH_2N_2, and then was vacuum chromatographed using a 10 cm glass vacuum cylinder (Silicar 76F-S TLC) with increasing polarity of solvent (EtOAc/isooctane). The methylated farlowdiols were eluted with 25% EtOAc/isooctane as determined by thin layer chromatography. Approximately 60 mg of the methylated farlowdiol mixture **34** and **35** was dissolved in 2 ml of pyridine and 2 ml of CH_2Cl_2, and then 50 mg of parabromobenzoyl chloride was added. The mixture was refluxed for 2 hours, and a second vacuum chromatograph was used to remove unreacted material from the bis-parabromobenzoate derivatives of methylated **34** and **35** (Silicar 76F-S TLC, EtOAc/isooctane increasing polarity). The bis-parabromobenzoate derivatives of the farlowdiol mixture were

separated by normal phase HPLC (Versa-pak, 50 cm x 4.1 mm, 4% EtOAC/isooctane) and were identified by spectroscopic analysis. Approximately 1.0 mg of the dibromobenzoyl derivative of compound **34** and 1.5 mg of the dibromobenzoyl derivative of compound **35** were produced (approx. 5% yield).

The hydrogenated dibromobenzoate derivative of the methylated farlowdiol **35** was prepared as follows. Approximately 1.5 mg of the bis-parabromobenzoate derivative of methylated **35** was diluted in ether containing platinum oxide and the mixture was saturated with hydrogen (11 psi) for 1 hour with constant stirring. The resulting material was then separated by normal phase HPLC (Versa pak, 50 cm x 4.1 mm, 3.0% EtOAc/isooctane) and the hydrogenated bis-parabromobenzoate derivative was analyzed spectroscopically.

Methyl 12,13-bis(p-bromobenzoyl)-5(Z),8(Z),10(E),14(Z)-icosatetraenoic acid **40**: UV λ_{max} 244nm ($\epsilon = 3.2 \times 10^4$); ^{1}H-NMR ($CDCl_3$) σ 7.87 (4H, m),7.58 (4H, m), 6.73 (1H, dd, J=11.1,14.1Hz), 6.07 (1H, dd, J=3.6,8.9Hz), 6.00 (1H, dt, J=11.0,11.0Hz), 5.77 (3H, m), 5.53 (2H, m), 5.36 (2H, m), 3.66 (3H, s), 2.91 (2H, bdd, J=6.9,7.1Hz), 2.30 (2H, t, J=7.6), 2.25 (2H, m), 2.09 (2H, dt, J=7.0,7.0Hz), 1.69 (2H, tt, J=7.4,7,4Hz), 1.40 (2H, m), 1.29 (4H, m), 0.89 (3H, t, J=6.9Hz); CD(MeOH) ϵ = 16.83 l/mol cm (253nm), 0.0 l/mol (240nm), -13.67 l/mol cm (233nm).

Methyl 12,13-bis(p-bromobenzoyl) 5(Z),8(Z),10(E),14(Z),17(Z)-icosapentaenoic acid **41**: UV λ_{max} 244nm ($\epsilon = 3.9 \times 10^4$); ^{1}HNMR

($CDCl_3$) δ7.87 (4H, dd, J=8.2, Hz), 7.57 (4H, dd, J=8.6Hz), 6.74 (1H, dd,J=11.1,14.1Hz), 6.09 (1H, dd, J=3.7, 8.9Hz), 6.00 (1H, dt, J=11.0,11.0Hz), 5.80 (1H, m), 5.74 (2H, m), 5.56 (1H, m), 5.50 (1H, m), 5.40 (1H,m), 5.31 (1H, m), 3.72 (3H, s), 3.00 (2H, bdd), 2.91 (2H, bdd, J=6.9,7.1Hz), 2.30 (2H, t, J=7.6Hz), 2.09 (2H, dt, J=7.0,7.0Hz), 1.68 (2H, tt, J=7.4,7.4Hz), 0.93 (3H, t, J=7.3Hz); CD(MeOH) ϵ = 24.10 l/mol cm (253nm), 0.0 l/mol cm (242nm), -21.75 l/mol cm (233nm).

Methyl 12,13 p-Dibromobenzoyl Dodecanoic Acid **42**:
UV λ_{max} 244 nm; ^1H NMR ($CDCl_3$) δ7.84 (4H, d, J=8.3Hz), 7.57 (4H, J=8.7Hz), 5.34 (2H, m), 3.66 (3H, s), 2.29 (2H, t, J=7.5Hz), 1.69-1.55 (8H, m), 1.40-1.15, (25H, m).

Results and Discussion

The diacetate-methyl ester derivative of farlowdiol A (**37**) was a colorless and optically active oil which gave a measurable M^+ - acetate peak by hr eims affording a molecular formula of $C_{25}H_{38}O_6$ (7^o unsaturation). The ir specturm for **37** showed an intense carbonyl stretch for multiple esters ($\nu_{C=O}$ = 1734cm^{-1}), the protonic consequences of which were readily observed in the ^1H nmr (Table III.1) and defined two acetates and one methyl ester. By ^{13}C nmr, the remaining four degrees of unsaturation were

presented as olefinic bonds (Table 1) and two of these formed a conjugated system (λ_{max} = 236nm).

The overall structure was readily approachable by ^{1}H-^{1}H COSY experiments (figure III.3, d-6-bz) which lead to the generation of two partial structures and accounted for all of the atoms in the molecule.

The first partial structure began with a sharp 2H triplet at d2.09, which was readily identified from comparisons with model compounds as belonging to the C-2 protons of a fatty acid, in this case present as a methyl ester. These were correlated to two high field methylenes (H_2-3) at d1.57 which were in turn adjacent to an allylic methylene at d1.91 (H_2-4). The adjacent C-5 olefin proton was at slightly higher field than any other olefin proton (d5.25). The other proton of this olefin was located at d5.29 and was itself correlated to a bisallylic methylene at d2.78 (H_2-7). This methylene was a triplet and was therefore coupled to one other proton, located at d5.38 from the COSY experiment. This olefin proton was coupled by 11.0 Hz to another olefin proton at d5.98, thus defining a cis geometry for this double bond. This latter proton was further coupled to another olefin proton at d6.83 (H-10). The H-10 proton was coupled to its olefin partner (d5.78) by 15.0 Hz and was therefore of trans geometry. In turn, this olefin proton was coupled to the ã proton of a secondary acetate located at d5.87, which was adjacent to another such ∝-acetoxy proton at d6.20. This partial structure terminated with

a correlation between the H-13 proton and a two proton olefin multiplet at d5.59.

The second partial structure began with a poorly defined methyl triplet which showed correlation to a 4H multiplet at d1.23 (H_2-19, H_2-18). This multiplet was further coupled only to a 2H multiplet at d1.29, thus defining H_2-17. The H_2-17 signal was in turn correlated to a doublet of triplets at d2.22 (H_2-16) and was adjacent to an olefinic proton d5.59 (H-15).

Combination of these spin system-derived partial structures accounted for all of the atoms in the molecule and could be put together in only a single manner, thus giving the overall structure of farlowdiol A (37). The stereochemistry of the C5-6 olefin was given by the ^{13}C nmr chemical shift of the adjacent bisallyic methylene. A value of d26.43 for this carbon atom defines both adjacent olefins as possessing the Z stereochemistry.[110] Similarly, the shift of d28.40 for the methylene adjacent to the C14-15 olefin defines the stereochemistry as also being Z. The absolute stereochemistry of the vicinal diol was examined in the following section.

Assignment of the Absolute Stereochemistry of the Farlowdiols

In order to determine the absolute stereochemistry of the two hydroxyl groups of each farlowdiol, we selected the exiton chirality method.[101] This method has been employed successfully

to deduce the absolute stereochemistry of both acyclic and cyclic compounds with one or more chromophores; these include glycols, terpenes containing hydroxyl groups and several hydroxyl-olefin-containing lipids.[101,102,103,104]

The CD spectrometer records the $\pi - \pi^*$ absorption of two chromophores that are located in chiral positions with respect to each other, and the resulting spectrum represents the stereochemistries of the excited chromophores. The exiton interaction between the two chromophores splits the excited state into two energy levels.[101] The two split energy levels generate Cotton effects of mutually opposite signs. This results in a curve having two extrema at longer and shorter wavelengths between the UV maxima of the chromophores; these extrema are known as the first and second Cotton effects, respectively.[101] If the electric transistion dipole moments of the two chromophores constitute a right-handed screwness, the sign of the first Cotton effect will be positive and the second, negative.[101] If the dipole moment has a left-handed screwness, the opposite sign for each Cotton effect will be observed.[101]

Since the hydroxyl groups of the farlowdiols are not suitable chromophores, the bis-parabromobenzoate derivative was produced. Also, the diene functionality contained in the farlowdiols has an absorption maximum near the maximum of the para-bromobenzoate, so the CD spectum of both the unsaturated and saturated bis-parabromobenzoate derivatives of the farlowdiols was recorded.

Since we observed a positive split Cotton effect for both of the bis-parabromobenzoate unsaturated derivatives we assume positive chirality for the two chromophores, which constitutes a right-handed screwness. This allows for 1 rotomer for each possible stereoisomer (Figure III.4). For the SS stereoisomer, the protons are separated by 180°, and we would expect a $J_{12,13}$ value around 9.0 Hz. Instead, we see $J_{12,13}$ = 3.6 Hz, so that the protons in this derivative are separated by approximately 60°, as is the case for the SS,SR and RS stereoisomers. However, the RS and SR stereoisomers have the bulky fatty acid chains separated by only 60°, which would be an unlikely conformation for an acyclic system. The RR stereoisomer has the bulky sidechains separated 180° apart and is thus the most likely stereoisomer of the four possibilities.

In order to exclude the possible effect of the diene chromophore coupling with the dibenzoate chromophores, we produced the completely saturated fatty acid derivative **42**. Circular dichroic analysis of compound **42** afforded no observable split Cotton effect. This derivative was not rigorously identified due to its low yield. There is a possibility that the diene chromophore may have obstructed the interpretation of the positive split Cotton effect observed in the two bis-parabromobenzoyl farlowdiol derivatives **40** and **41**, and that the assignment of RR for the two hydroxyl functionalities is incorrect. In order to prove the absolute stereochemistry of the farlowdiols, more experimentation will be necessary.

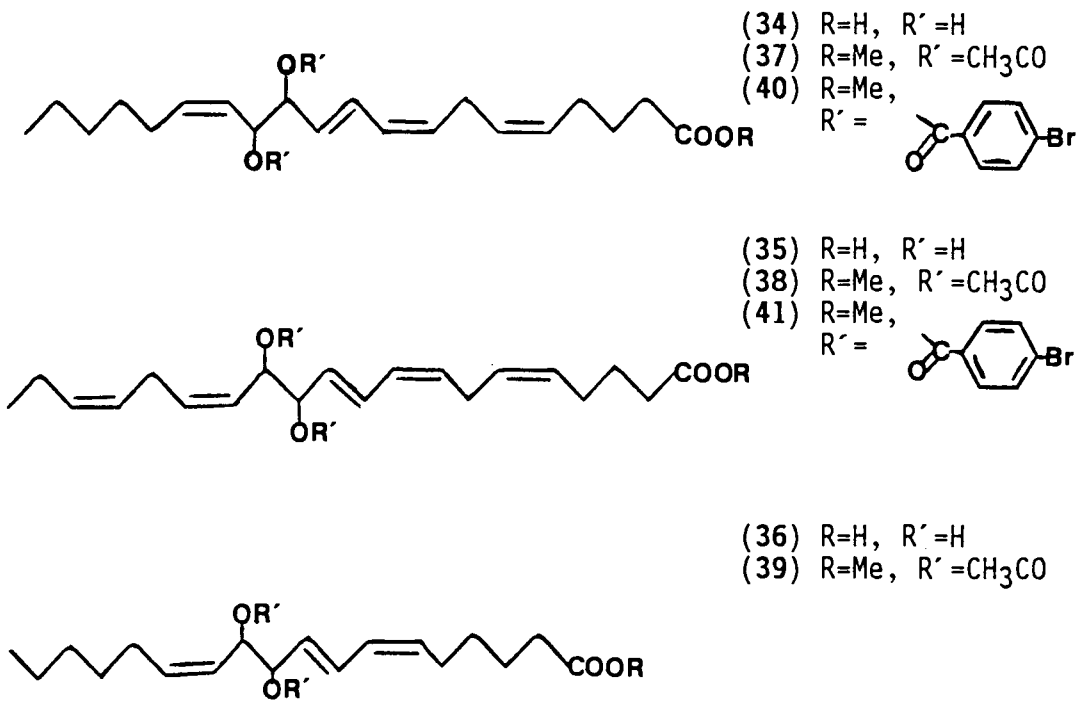


Figure III.1 Structures of the farlowdiols and their derivatives

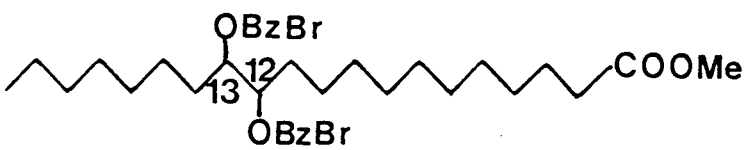


Figure III.2 Structure of the hydrogenated bis-para-bromobenzoate derivative 42

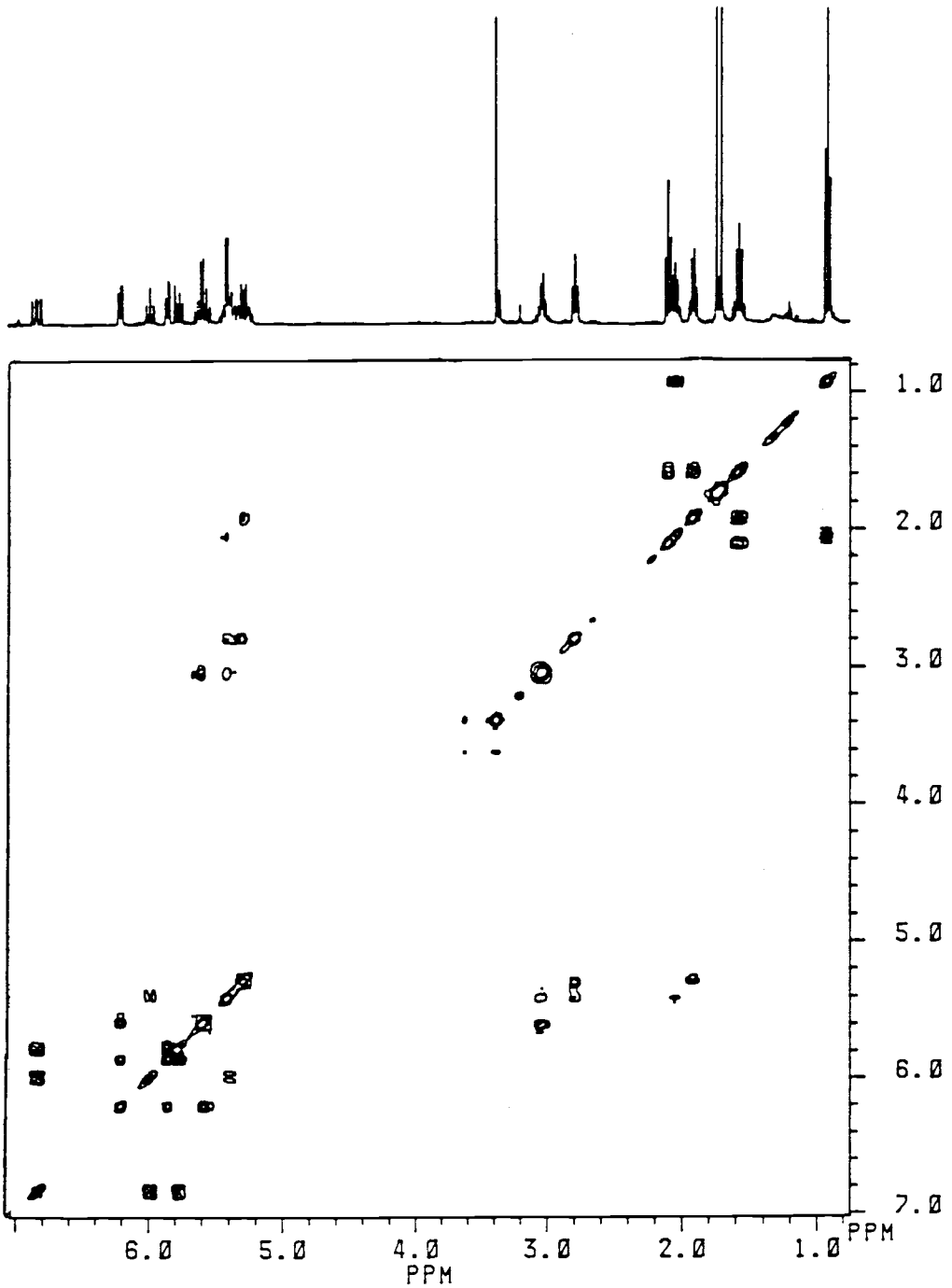


Figure III.3 ^{1}H-^{1}H COSY of 12,13-diacetoxy,5(Z),8(Z), 10(E),14(Z),17(Z) icosapentaenoic methyl ester (38) showing correlations between coupled protons (ca. 3.5 mg of (38) in 0.4 ml deuterated C_6H_6 with 0.3% TMS, 5.0 mm tube, 400 MHz)

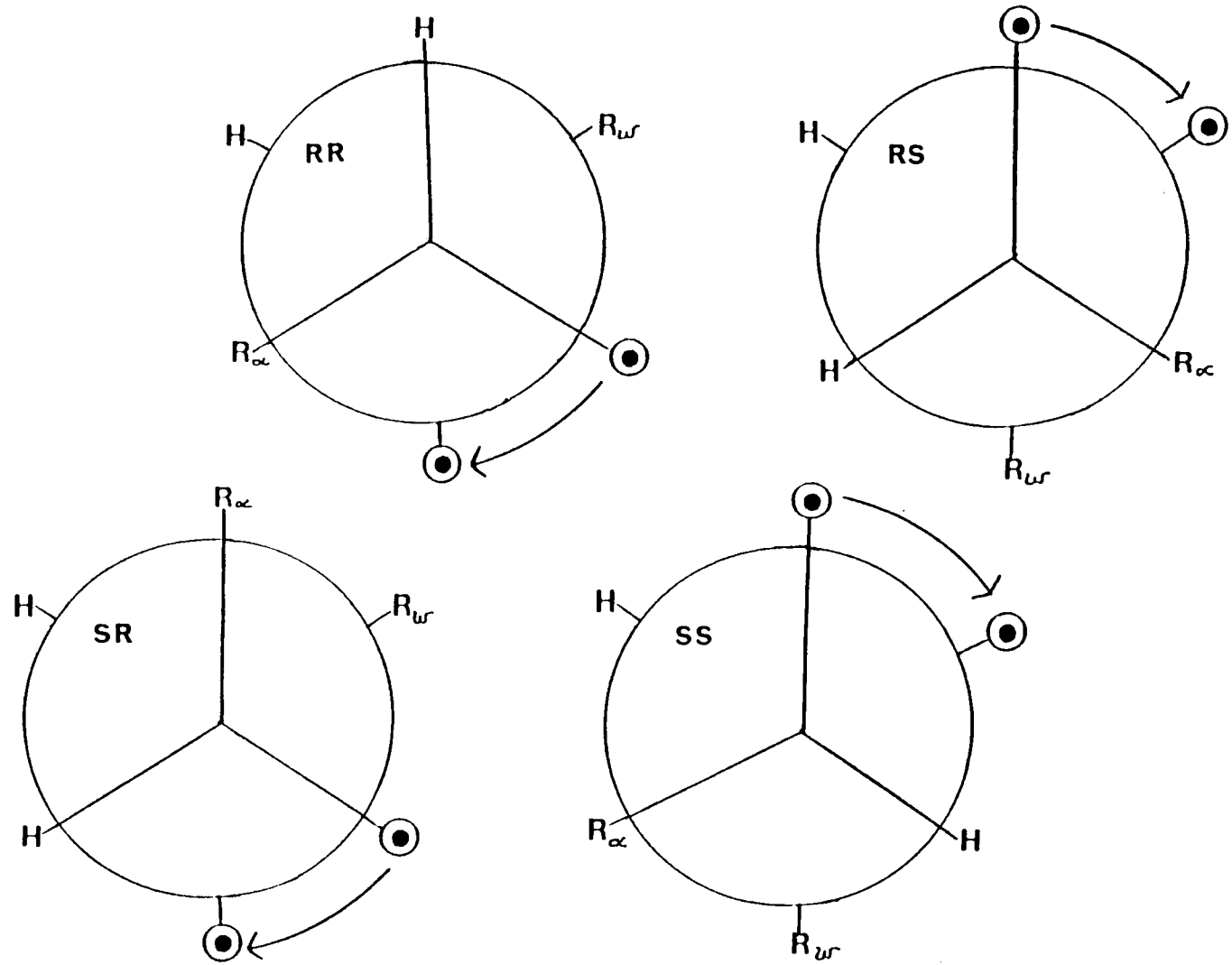


Figure III.4 Four possible stereochemical conformations for the farlowdiol derivatives 40 and 41

Table III.1
NMR Data for the Methyl Ester Diacetate Derivatives of three Icosanoid Natural Products from Farlowia mollis[a]

C	Compound 37				Compound 38				Compound 39			
	^1H			^{13}C[b]	^1H			^{13}C[c]	^1H			^{13}C[d]
No.	δ	m	J(Hz)	δ	δ	m	J(Hz)	δ	δ	m	J(Hz)	δ
1	-	-	-	173.18	-	-	-	173.18	-	-	-	e
2	2.09	t	7.3	33.32	2.09	t	7.3	33.31	2.01	t	7.4	33.85
3	1.57	tt	7.3,7.3	25.03	1.57	tt	7.3,7.3	25.02	1.43	tt	7.4,7.6	24.65
4	1.91	dt	7.3,7.3	26.75	1.91	dt	7.3,7.3	26.75	1.15	tt	7.2,7.6	29.13
5	5.25	m	-	129.86	5.25	m	-	129.86	1.92	dt	7.2,7.2	27.60
6	5.29	m	-	128.16	5.29	m	-	e	5.31	m	-	134.44
7	2.78	bdd	7.3,7.3	26.43	2.77	bdd	7.2,7.2	26.42	5.97	dtt	11.0,-	127.93
8	5.38	dt	11.0,7.3	132.44	5.40	m	-	132.48	6.75	dd	11.0,15.0	130.91
9	5.98	dt	11.0,11.0	127.93	5.97	bdd	11.2,11.2	127.90	5.78	dd	8.1,15.0	127.04
10	6.83	dd	11.0,15.0	130.75	6.82	dd	15.2,11.2	130.84	5.87	dd	3.7,8.1	75.61
11	5.78	dd	15.0,8.2	127.38	5.76	dd	15.2,8.2	127.23	6.21	dd	3.7,8.2	70.65
12	5.87	dd	8.2,3.7	75.58	5.85	dd	8.2,3.8	75.47	5.60	m	-	124.02
13	6.20	dd	3.7,8.0	70.61	6.19	dd	3.8,8.5	70.54	5.60	m	-	136.91
14	5.59	m	-	124.02	5.55	m	-	124.13	2.21	m	-	28.40
15	5.59	m	-	136.93	5.59	m	-	135.02	1.30	m	-	29.46
16	2.22	m	-	28.40	3.03	m	-	26.75	1.25	m	-	31.71
17	1.29	tt	6.9,6.9	29.47	5.40	m	-	126.31	1.22	m	-	22.87
18	1.23	m	-	31.72	5.40	m	-	133.04	0.89	t	6.8	14.22
19	1.23	m	-	22.89	2.03	dq	7.3,7.3	20.88				
20	0.86	t	6.8	14.22	0.91	t	7.3	14.38				
1'	3.65	s	-	50.98	3.65	s	-	50.99	3.65	s	-	50.98
Ac1	1.70	s	-	20.64	1.70	s	-	20.69	1.70	s	-	20.65
	1.75	s	-	20.70	1.75	s	-	20.69	1.70	s	-	20.65
Ac2 (2C)	-	-	-	169.51	-	-	-	169.46	-	-	-	169.46

[a] Chemical shift values in ppm relative to TMS as an internal standard operating at 9.398. All spectra obtained in deuterated benzene.
[b] Assignments made from a ^1H-^{13}C heteronuclear 2D shift correlation spectroscopic experiment and by comparison with model compounds (1,2).
[c] Assignments made by comparing with (37) and with model compounds (112,109).
[d] Assignments made by comparing with (37),(38) and with model compounds (12,109).
[e] Peak not observed in spectrum.

CHAPTER IV. Production of Superoxide Anion by Human Neutrophils Stimulated with Novel Fatty Acids from Farlowia mollis

After the structures of the novel fatty acid diols from Farlowia mollis had been elucidated, we noted their structural similarities to several biologically active icosanoids produced in mammalian systems, particularly the lipoxins and several DHETEs. We decided to test these compounds for the presence of a common biological activity of stimulating the oxidative metabolism in human neutrophils.

A biological property that many of the lipoxygenase-derived icosanoids share is the ability to either independently stimulate or augment the production of superoxide anion by human neutrophils.[43,44] LTB_4 and LXA have been shown to be independent stimulators of O_2^- production at levels comparable to fMLP.[43] In other experiments, LTB_4 was found to be a weak stimulator of superoxide production, but was found to greatly enhance O_2^- production in cells stimulated with fMLP.[79] Also, other DHETEs and HETEs have been tested, and have been shown to augment superoxide production by neutrophils stimulated with fMLP, although the fatty acid concentrations needed were 10 to 100 times greater than that of LTB_4.[44]

It is known that arachidonate and other non-hydroxylated

fatty acids can independently stimulate superoxide production.[69,70] But, the lipoxygenase-derived fatty acids are much more potent mediators.[44] Some icosanoids have been found to inhibit the stimulation of superoxide production by fMLP or LTB_4.[46,72] The prostaglandins, PGE_1 and PGI_2, have demonstrated a 50% or greater inhibition of fMLP induced O_2^- production at concentrations of 10 m.[72] Also, 14,15-DHETE can inhibit LTB_4-induced O_2^- production by human neutrophils at similar concentrations, although it has no inhibitory effects on fMLP-induced superoxide production.[46] We decided to test the novel fatty acid diols isolated from Farlowia mollis, as well as a few other fatty acids isolated from marine algae in our laboratory, for their effects on the oxidative metabolism in human neutrophils.

Methods[55]

Approximately 150 to 200 ml of whole blood (ACD anti-coagulant) was drawn from various donors by certified members of the Red Cross or Good Samaritan Hospital (Corvallis, Oregon). The blood was immediately subjected to dextran precipitation (6% Dextran, M.W.=70,000, Pharmacia). Approximately 60 mls of whole blood were mixed with 40 mls 6% Dextran, and the cells were allowed to stand for 1 hour, undisturbed, in a 100 ml plastic graduated cylinder. After this time, most of the RBCs settled to the bottom, leaving a straw colored, leukocyte rich plasma on top.

This plasma was drawn off and centrifuged at 120 x g for 20 minutes at 4°C. The supernatant was discarded, and the cells were shocked with ice cold distilled H_2O (approx. 1/10 the volume of the original plasma suspension), and after 30 seconds their isotonicity was restored by the addition of 0.6M NaCl (1/3 the volume of distilled H_2O). The cells were washed with PBS (Sigma, w/o Ca^{+2}), and were subject to a discontinuous gradient centrifugation using Ficoll-Paque (Pharmacia). Approximately $3x10^7$ cells in 8 ml PBS were layered onto 4 ml Ficoll-Paque in a 15 ml glass centrifuge tube, and were centrifuged at 250 x g for 20 minutes at 4°C. The pellet of cells at the bottom of the tube contained approximately 99% PMNLs. These cells were washed with PBS, then were resuspended using Hank's buffered saline soution (Sigma, with Ca^{+2}, w/o phenol red (HBSS)) at a concentration of 6.0×10^6 cells/ml. The cells were counted using a hemocytometer and a light microscope. The viability of the cells was determined using a trypan blue exclusion assay in which only damaged cells take up the dye. A solution of trypan blue (0.5%, in PBS) was mixed (1:1) with the cells, and was incubated at room temperature for 5 minutes. At the end of this time, the cells were examined under a light microscope for the uptake of the dye. In all of the assays less than 5% of the cells took up the dye. All exposed glassware and other materials were disposed of using guidelines set up by O.S.U. procedures for handling and disposing of human blood.

Superoxide Anion Assay

The following protocol was used to measure the production of superoxide anion by isolated human neutrophils.[44,72] In each experiment, cells were isolated for 1-2 hours before the start of the assay. Isolated neutrophils (3-4 x 10^6/0.5 ml Hank's buffered saline solution (HBSS)) were incubated for 10 minutes at 37°C. Following this, cytochalasin B (5μg/ml) was added to the cells and incubated for 3 minutes. If no cytochalasin B was used, the incubation time of 3 minutes was still allowed. Test lipids (0.10 - 10μM) were then added to the cells, and the mixture was incubated at 37°C for 2 minutes. Next, cytochrome C (final conc. 10 mM in HBSS) was added to the mixture. If fMLP or LTB_4 were to be tested as an additional stimulus, these agents were then added to the cell mixture. The mixture was then incubated at 37°C for 15 minutes. All assays had a final volume of 2.0 ml. After the incubation period, the reaction was stopped by the addition of 10 μl of superoxide dismutase (1 mg/0.3 ml HBSS). This enzyme converts the superoxide anion to hydrogen peroxide, preventing any further reduction of cytochrome C. The cells were then pelleted by centrifugation at 200 x g for 10 minutes. The supernatant was measured for absorbances between 500-570 nm using an Aminco spectrophotometer. The maximum absorbance of reduced cytochrome C is at 550 nm. Blanks were used to give baseline values. Before cytochalasin B, fMLP or LTB_4 were added to the cells, 10 μl of SOD

would be introduced for each control. The incubation period was the same as for all other assays. The amount of superoxide was determined using the following equations:[65]

$$\text{(Absorb. at 550nm,sample)} - \frac{\text{(Absorb. at 540nm + 560nm,sample)}}{2} -$$

$$\text{(Absorb. at 550nm,blank)} - \frac{\text{(Absorb. at 540nm + 560nm,blank)}}{2} = A$$

$$O_2^- \text{ (nmoles)} = A \times \text{(vol. of assay, ml)} \times 47.4$$

Table IV.1 gives the values assigned for O_2^- production in different experimental conditions of stimulated neutrophils. Values are in nmoles/# cells, and can be compared to published experimental values in tables IV.2 and IV.3.

The solvent used to dissolve the fatty acids and fMLP was 95% EtOH diluted to less than 0.25% using HBSS. This amount of EtOH has no effect on cell activity.[67] To dissolve cytochalasin B, dimethyl sulfoxide was used (final volume in the assay was 0.2%). This amount of DMSO has no effect on the oxidative metabolism of neutrophils.[81]

Results and Discussion

Tables IV.1, IV.2 and IV.3 presents the values recorded for the production of superoxide anion by neutrophils under various experimental conditions. The experimental design closely

resembles the protocol used in references 44 and 72, so that the data can be directly compared to the results of these experiments.

Donors 1, 2 and 3 (Table IV.1) all show a similar response in their neutrophil activity when stimulated with 10^{-7}M fMLP, with an average of approximately 20 nmoles O_2^- generated by 3 x 10^6 cells after a 15 minute incubation period. Also, the presence of cytochalasin B augmented the production of O_2^- induced by fMLP in all three donors, although the magnitude of the augmentation varied between donors. This augmentation is believed to be due to a continuous occupancy of the peptide at the receptor site in the presence of cytochalasin B, which gives a prolonged response by the stimulated cells.[81,105] Also, cytochalasin B decreases the lag time of superoxide production in cells stimulated with the peptide.[105] However, neutrophils from donor 1 showed an inhibition of O_2^-production by cytochalasin B in the presence of several different icosanoids (LTB_4, the farlowdiols and 12-HEPE). This effect by the fungal metabolite may suggest an inhibition of a pathway leading to the production of superoxide anion that is separate from fMLP. Donor 1 also showed an unusually sensitive response to LTB_4, the farlowdiols and other icosanoids tested in the absence of cytochalasin B and fMLP.

The response of the various donors to LTB_4 was variable. In donor 1 there was a moderate amount of superoxide anion generated by the compound, whereas donors 2 and 3 showed little or no superoxide anion production under the same experimental conditions. Variations in the magnitude of LTB_4 stimulation of

neutrophil activity can be seen in the literature for superoxide production as well as for aggregation and degranulation.[34,49] This may be due to the variability in experimental conditions, including the buffer composition, the presence and concentration of cytochalasin B, the incubation period and the variability in the different donor's cell activities.

Although the triene mixture (see reference 12, compounds (5),(6)) was not rigorously tested, it appears from the data that these compounds are weak stimulators of superoxide production and can augment this response in cells stimulated with fMLP. The compound 12-HEPE (reference 27, compound (8)), which was isolated by our laboratory from the Puerto Rican red alga Murrayella periclados, also appears to be a stimulator of O_2^- production and an augmenter of O_2^- production in fMLP-stimulated cells. Further experimental observations will determine whether the magnitude of these responses are significant.

A mixture of the 20-carbon fatty acid diols from Farlowia mollis (34) and (35) was tested in several different experimental conditions. These diols appear to be weak primary stimulators of superoxide production. However, a fairly prominent response was seen in the cells from donor 1 in the presence of Ca^{+2} (Table IV.1). Other fatty acids tested with the PMNLs from donor 1 also showed a greatly enhanced stimulatory activity when compared to the other donors and other literature values for various icosanoids as independent stimulators of superoxide anion generation. This hyperactive response is not explained, however,

some of this response may be due to the presence of anti-inflammatory drugs, including ibuprofin, in the donor's system at the time the blood was drawn.

The Farlowia diols (**34**) and (**35**) were also tested at different concentrations in the presence of fMLP. At 10^{-5}M, all three donors showed an inhibition of fMLP-stimulated superoxide anion production that ranged between 15% and 34% of control values. This inhibitory activity is comparable to the activities of the prostaglandins PGE_1 and PGI_2 at equivalent concentrations (Table IV.2). However, at lower concentrations, these diols had activities similar to other DHETEs tested using fMLP-stimulated cells. The farlowdiols showed a mild augmentation of superoxide production in neutrophils (Table IV.3).

Overall, the Farlowia diols appear to be modulators of superoxide production by human neutrophils. The molecular mechanism of their effect on cell activity is not known. Other diol-containing icosanoids have been shown to inhibit lipoxygenase activity.[107] Preliminary data on the Farlowia diols suggest an inhibition of 5-lipoxygenase activity (38% inhibition at 10^{-4} M).[108] If this inhibition by the farlowdiols is related in any way to the inhibition of superoxide production by fMLP-stimulated neutrophils remains to be determined.

Table IV.1. Superoxide Anion Production in Human Neutrophils Stimulated by Various Icosanoids and Other Compounds

(measured in nmoles O_2^-/3.0x10^{6}cells)

Conditions	Donor 1 no Ca^{+2} (3.0x10^{6}cells)	Donor 1 Ca^{+2} (3.0x10^{6}cells)	Donor 2 Ca^{+2} (3.0x10^{6}cells)	Donor 3 Ca^{+2} (3.0x10^{6}cells)
1.Control (SOD,fMLP) w/o CytoB	ND	ND	ND	ND
2.Control (SOD,fMLP) w CytoB	ND	ND	ND	ND
3.Control (SOD,LTB_4)	ND	ND	ND	ND
4.Control (cells,no SOD)	-	-	-	ND
5.fMLP (10^{-7}M)	20.3	20.8	20.4(2)	14.6(2)
6.fMLP (10^{-7}M) w CytoB	-	90.5	29.8(2)	42.7(2)

7. LTB_4 (10^{-7}M) LTB_4 (10^{-8}M)	8.4 5.9	13.0 -	2.8 -	1.1(2) -
8. LTB_4 (10^{-7}M) w CytoB	-	4.0	ND	0.9(2)
9. Farlowia DHET(P)E (10^{-5}M)	8.2	19.4	2.1	5.5(2)
10. Farlowia DHET(P)E (10^{-6}M)	-	22.2	ND	2.7(2)
11. Farlowia DHET(P)E (10^{-7}M)	-	23.7	1.4	2.6(2)
12. Farlowia DHET(P)E (10^{-5}M), CytoB	-	2.6	1.3	ND(2)
13. Farlowia DHET(P)E (10^{-6}M), CytoB	-	-	-	ND(2)

Table IV.1 (continued)

14. *Farlowia* DHET(P)E (10^{-7}M), CytoB	-	5.8	3.3	ND(2)
15. *Farlowia* (10^{-5}M) fMLP (10^{-7}M)	32.2	17.5	17.1	9.5(2)
16. *Farlowia* (10^{-6}M) fMLP (10^{-7}M)	-	24.7	-	-
17. *Farlowia* (10^{-7}M) fMLP (10^{-7}M)	26.9	21.8	21.8	16.7(2)
18. LTB_4 (10^{-7}M) fMLP (10^{-7}M)	-	-	26.4	14.2(2)
19. Cyto B (5 g/ml)	-	1.4	5.6	ND(2)
20. *Farlowia* (10^{-5}M) LTB_4 (10^{-7}M)	6.5	-	ND	0.8(2)

Table IV.1 (continued)

21. *Farlowia* (10^{-7}M) LTB_4 (10^{-7}M)	4.1	-	ND	2.5(2)
22. *Farlowia* (10^{-5}M) LTB_4 (10^{-8}M)	8.5	-	-	-
23. *Farlowia* (10^{-7}M) LTB_4 (10^{-7}M)	6.1	-	-	-
24. triene mix* (10^{-5}M)	14.1	-	-	-
25. triene mix (10^{-7}M)	3.3	-	-	-
26. triene mix (10^{-5}M) fMLP (10^{-7}M)	34.1	-	-	-
27. triene mix (10^{-7}M) fMLP (10^{-7}M)	28.5	-	-	-

Table IV.1 (continued)

28.12-HEPE methyl ester				
(10^{-6}M)	9.4	-	-	-
(10^{-7}M)	3.3	-	-	-
29.12-HEPE	-	17.1	-	-
(10^{-6}M)				
(10^{-7}M)	-	20.9	-	-
30.12-HEPE,CytoB	-	3.8	-	-
(10^{-6}M)				
(10^{-7}M)	-	4.7	-	-
31.12-HEPE	-	35.1	-	-
(10^{-6}M)				
(10^{-7}M)	-	17.1	-	-
fMLP (10^{-7}M)				
32.12-HEPE	-	24.7	-	-
(10^{-6}M)				
(10^{-7}M)	-	21.8	-	-
LTB_4 (10^{-7}M)				

* approximately a 1:1 mixture of the cis,trans,trans and trans,trans,trans fatty acids found in Ptilota filicina, Farlowia mollis and Mureyella periclados. See compounds 5,6 or ref. 12.

ND = none detected, - = no experimental data

Table IV.1 (continued)

Table IV.2: Inhibition of fMLP Induced Neutrophil O_2^- Production by Various Icosanoids

Compound	O_2^- nmoles/3.0x10^{6}cells	%inhibition
1. fMLP (10^{-7}M)*	28.3	
2. + 15-M PGE_1* ($4x10^{-5}$M)	4.7	83.6
3. + 15-M PGE_1* (10^{-5}M)	11.4	59.8
4. + PGI_2 * ($3.0x10^{-5}$M)	14.8	47.7
5. fMLP (10^{-7}M) (donor 1, w Ca^{+2})	20.8	
6. + Farlowia DHET(P)E (10^{-5}M)	17.5	15.9
7. fMLP (10^{-7}M) (donor 2, w Ca^{+2})	20.4(2)	
8. + Farlowia DHET(P)E (10^{-5}M)	17.1	16.2

9. fMLP		
(donor 3, w Ca^{+2})	14.6(2)	
10. + *Farlowia*		
DHET(P)E		
(10^{-5}M)	9.5(2)	35.0

* data from reference 72

Table IV.2 (continued)

Table IV.3 Effects of Selected Icosanoids on the O_2^- Production by Neutrophils Treated with fMLP

Agent	fMLP induced O_2^- (% of control) $10^{-6}M$	$10^{-7}M$
1. 5-HETE*	175 (4)	152 (3)
2. 14,15-DHETE*	134 (4)	111 (3)
3. 12-HETE*	161 (2)	115 (2)
3. triene mix	-	140
4. 12-HEPE	169	83
5. Farlowia DHET(P)E	119**	105**
		107(2)***
		114(2)****

*data from reference 44
**data from donor 1, w/o Ca^{+2}
***data from donor 2, w Ca^{+2}
****data from donor 3, w Ca^{+2}

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