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ECOLOGY OF CHEMICAL DEFENSES OF ALGAE
AGAINST THE HERBIVOROUS SNAIL, LITTORINA LITTOREA,
IN THE NEW ENGLAND ROCKY INTERTIDAL COMMUNITY

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by

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ABSTRACT

In the New England rocky intertidal community, space is dominated by two perennial plant types, brown furoid algae (Ascophyllum nodosum and several species of Fucus) in the mid zones and the red alga Chondrus crispus in the low zones. These algae are not grazed by the predominant herbivorous snail, Littorina littorea. Here I report the first direct evidence that these algae produce chemicals which inhibit feeding by the snails.

Polyphenols in Fucus vesiculosus and Ascophyllum nodosum were shown to be effective chemical defenses against the snails. Feeding experiments demonstrated that the presence in the diet of as little as 1% polyphenol (dry weight), extracted from these two algal species, caused a significant reduction in feeding by L. littorea; 10% polyphenol (dry wt.) in food media inhibited snail feeding nearly 100%. The phenol and polyphenol contents in different tissues of these two algal species and

in other New England rocky intertidal algal species were monitored monthly for one year. F. vesiculosus and A. nodosum showed highest polyphenol contents (1-17% dry wt.); these levels were sufficiently high in all tissues during all months to inhibit snail feeding. The mechanism of action of plant polyphenols against herbivores is through their binding to plant proteins and other nitrogenous compounds, rendering them indigestible. Polyphenol contents were therefore examined in relation to plant nitrogen contents (using polyphenol/nitrogen ratios) to estimate the unavailability of plant nitrogen to herbivores due to polyphenol binding.

Annual brown algal species such as Petalonia fascia and Scytosiphon lomentaria had significantly lower levels of phenols and polyphenols than the perennial F. vesiculosus and A. nodosum. These two species are highly preferred as food by L. littorea. C. crispus and the green alga Codium fragile also had low phenol and polyphenol levels, yet they are of low food preference to the snails. Methylene chloride extracts from C. crispus and volatile halocompounds from C. fragile inhibited snail feeding, hence these species have chemical defenses quite different from those of F. vesiculosus and A. nodosum. Factors such as physical defenses, nutritional content, and temporal and spatial escapes are also important in determining algal food preference to herbivores .

The release into seawater of volatile hydrocarbons and halomethanes from benthic algae and seagrass was measured to examine the possible role of these compounds as antiherbivore compounds. Bioassays indicated that CH_2I_2 , a compound released into seawater from C. fragile, inhibited

feeding of L. littorea. CHBr_3 , released into seawater by many algal species, appeared to have less activity against the herbivores.

This study represents one of the first examinations of plant chemical defenses against herbivores in the marine environment. The findings are discussed in relation to recent theories from terrestrial studies on the commitment of plants to chemical defense.

Name and Title of Thesis Supervisor: John M. Teal, Senior Scientist

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PREFACE

This thesis is composed of a general introduction and five chapters dealing with the ecology of algal chemical defenses against a marine herbivore. Chapters are written as discrete papers, each with introduction, methods, results, discussion and conclusion.

The general introduction to the thesis reviews the literature on plant chemical defenses in terrestrial and marine environments. It has been well documented in terrestrial environments that plants produce many diverse chemicals that deter feeding by invertebrate and vertebrate herbivores. Although many unusual natural products have been isolated from marine algae, and some have been shown toxic to bacteria, phytoplankton, fungi, fish, and insects, few have been tested for an antifeeding function against marine herbivores found in the environment of the alga from which they were extracted. The marine algae and periwinkles of the New England rocky intertidal community are suggested to comprise a suitable system in which to investigate the possible existence and importance of algal chemical defenses against herbivores.

Chapter 1 documents the first evidence for chemical defenses in several species of marine algae (Fucus vesiculosus, Ascophyllum nodosum, Chondrus crispus, and Codium fragile) against herbivores (Littorina littorea) in the New England rocky intertidal community. A bioassay was developed to determine the effect of algal compounds on the feeding of periwinkle snails.

In Chapter 2, the antiherbivore compounds in F. vesiculosus and A. nodosum are identified as polyphenols and their effective doses (ED₅₀) against L. littorea are determined. This work was done in cooperation with Dr. Oliver McConnell, Chemistry Department, Skidaway Institute of Oceanography (I carried out all bioassays of the algal extracts and compounds with L. littorea while Dr. McConnell performed all molecular weight determinations and spectroscopic analyses of the compounds). I concluded that polyphenols in F. vesiculosus and A. nodosum are functionally similar to terrestrial plant polyphenols (tannins) in their roles as chemical defenses against herbivores.

Chapter 3 reports the polyphenol contents in thirteen species of brown, red, and green algae in the New England rocky intertidal community. The seasonal and within-plant variations in polyphenol content in F. vesiculosus and A. nodosum are examined. Results are discussed in relation to the grazing patterns of L. littorea, the predominant macroalgal grazer in this New England rocky intertidal community.

In Chapter 4, the seasonal and within-plant variations in algal nitrogen content are examined in relation to the algal polyphenol contents reported in Chapter 3. The effect of algal polyphenols on nitrogen availability to herbivores is discussed and comparisons are drawn between brown algae and terrestrial plants in the nature and functioning of chemical defenses against herbivores.

Chapter 5 identifies volatile compounds produced by marine algae and seagrasses in New England intertidal and subtidal communities and

examines their roles in algal chemical defense against herbivores. Part I is a paper (submitted to Journal of Phycology) that I coauthored with Philip Gschwend. It describes work we did in collaboration at Woods Hole Oceanographic Institution (I surveyed and identified all plant species in our study sites, assisted in the batch incubations of the samples, and interpreted the ecological roles of the algal compounds; Dr. Gschwend identified the algal compounds using gas chromatography/mass spectrometry and assessed the release rates of the compounds into seawater). We concluded that saturated and unsaturated hydrocarbons and di- and trihalomethanes were released by the algae at rates that may account for the concentrations of these volatile organic compounds in coastal seawater, and we hypothesized that some of these algal compounds may be important in allelochemic interactions. In Part II, I determined the effect of two of the volatile halomethanes (CH_2I_2 and CHBr_3) on feeding by L. littorea. CH_2I_2 , a compound released by C. fragile and F. vesiculosus, was shown to inhibit feeding by L. littorea. In contrast, CHBr_3 , a compound released by many algal species, did not significantly affect L. littorea feeding.

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I. Statement of the Problem

Plant-herbivore interactions along with predation and competition provide most of the organization of community structure in both terrestrial and marine environments. Comparisons between communities are useful in determining whether similar mechanisms act to produce similar patterns. In terrestrial systems, it has been well documented that plants produce chemicals which act as defenses against herbivores and affect their feeding behavior (Fraenkel, 1959; Whittaker, 1970; Schoonhoven, 1972; Feeney, 1976). Indeed, a general theory of chemical coevolution between higher plants and their herbivores has been proposed by Ehrlich and Raven (1964) and Feeny (1975) which provides a partial explanation of the observed patterns of interaction. The evolution of defensive plant compounds and the stepwise evolutionary responses to these by phytophagous organisms are postulated to have been dominant factors in the evolution of both the herbivores and the plants and thus in the generation of terrestrial diversity.

In marine systems, direct evidence for algal chemical defenses has been lacking. Although many natural products have been isolated from marine algae (Baker and Murphy, 1976; Faulkner and Anderson, 1974; Fenical, 1975; Scheuer, 1973), very little is known about the biological activity of these compounds. A few of these compounds have been shown to have antibacterial or antialgal effects (Bhakuni and Silva, 1974; McLachlan and Craigie, 1966; Sieburth and Conover, 1965), but their ecological importance in plant-herbivore interactions has

largely been ignored. This research will investigate experimentally the importance of the organic compounds in algae as a means of defense against herbivores.

The intertidal algae and periwinkles (in particular, Littorina littorea) of the New England rocky intertidal community comprise a particularly conducive system in which to study this problem for the following reasons; 1) the snails and algae are readily accessible in the field and can be maintained under laboratory conditions; 2) Littorina littorea, the most abundant herbivore in the community, is a generalist which feeds on a wide variety of macroscopic and microscopic algae and exhibits a definite performance regime for particular algal species, avoiding some entirely; 3) a variety of compounds such as tannins and halogenated metabolites in these algae have demonstrated antibacterial and antialgal activity and hence are suspected as antiherbivore defenses; 4) the algae of this community include species in the Rhodophyta, Chlorophyta, and Phaeophyta; their life histories vary, including ephemerals, annuals, perennials, and species with alternating stages; 5) the importance of L. littorea in determining the distribution, abundance, and diversity of algae in the community has been established; feeding preferences of the snails and defenses of the algae together form the underlying cause of the patterns observed (Menge, 1975). The questions of what determines the feeding preferences of the snails and what constitutes the mechanism of defense of the algae remain unanswered. The role of algal

antiherbivore compounds is unexplored in this community as throughout most marine communities.

The questions which I will investigate in this thesis include:

1) Do the algae which are not eaten by L. littorea, in particular the dominant fucoids and perennial reds, possess compounds that act as deterrents to grazing? Is inhibition of feeding due to these antiherbivore chemicals rather than to size or toughness of the algae? To which classes of compounds do these compounds belong? What are their structures?

2) Do the algae vary in the production of antiherbivore chemicals during different stages of their life cycles, with age or size, or in different tissues? Are these seasonal variations? Do these variations result in differential palatability to herbivores?

3) What patterns emerge between the production of algal antiherbivore chemicals and the apparency or successional status of the algae in the community? Within each algal taxonomic division, is commitment to chemical defense greater in perennials than in annuals and ephemerals as postulated by Gates and Orians (1975) for terrestrial plants?

This study combines the approaches of natural products chemistry and ecology to understand mechanisms of chemical ecology in the marine environment. Through comparisons to terrestrial studies this research will be useful in determining if similar mechanisms operate to produce similar patterns across marine and terrestrial communities.

II. Plant Chemical Defenses: Parallels Suggested Between Terrestrial and Marine Environments

A. Defenses of Plants Against Herbivores

Hairston, Smith and Slobodkin (1960) argued that because herbivores do not eat all plants and because populations of herbivores frequently increase greatly when predators are removed, herbivore populations must be limited by predators. This logic has serious flaws which were first pointed out by Murdoch (1966). The mere presence of plants in what appears to be unlimited supply does not necessarily mean that herbivores are not food-limited. Plants have evolved various defense systems which limit their availability to herbivores. Five types of plant antiherbivore strategies can be outlined as follows: 1) production of chemicals that are noxious or that decrease the quality of food, 2) mechanical and structural adaptations in the plant; 3) spatial escapes, 4) temporal escapes, and 5) size escapes. Because the production of defensive chemicals is so widespread and varied among plants, it has been considered the primary response to herbivore grazing pressure (Ehrlich and Raven, 1964). Stebbins (1950) labelled the other strategies as more specialized secondary responses evolving either in the absence or substantial failure of defensive compounds to discourage predation. A plant's best strategy against herbivores is to become inedible; this is well accomplished by the synthesis of metabolic compounds which are, for one reason or another, unacceptable to herbivores (Dethier, 1970).

B. Antiherbivore Chemicals in Terrestrial Plants

Allelochemicals, as defined by Whittaker and Feeney (1971) are chemicals by which organisms of one species affect the growth, behavior, or population biology of another species (excluding substances used only as food by the second species). Within this group are 1) allomones, which give adaptive advantages to the producing organism, 2) kairomones, which give adaptive value to the receiving organism, and 3) depressants, which are wastes which harm the recipient but which have no adaptive value to the releaser. Antiherbivore chemicals are thus allomones in this scheme. Allomones may serve as defenses against herbivores as well as against parasites, bacteria, and competing plant species; one compound may have specific or multiple roles (Muller et al., 1964; Muller and Muller, 1964; Wells, 1964; Levin, 1971). Thus, adaptive advantage and selective preservation of certain plant chemicals cannot be evaluated solely in terms of herbivores. It is important to remember that the effects of herbivores on evolution of plant chemical defenses must be viewed against a broader coevolutionary background.

The chemicals involved in defending plants from herbivores generally belong among the secondary compounds, as do most plant allomones. They are not, in most cases, essential to the basic metabolism of the plant and hence are contrasted with primary substances such as proteins, carbohydrates, fats, and nucleic acids. Secondary compounds may be wastes or elaborated by-products of various synthetic pathways. The nutritive value of plants to herbivores is basically determined by

the primary metabolites; the secondary compounds are thus said to function as attractant or repellent triggers (Fraenkel, 1959). Dethier (1947) and Schoonhoven (1972) compiled extensive lists of plant secondary compounds known to act as feeding stimulants or deterrents to insects. They are of irregular or sporadic occurrence, reinforcing the view that they are not of primary metabolic concern to plants. The occurrence of the same or related secondary compounds in related plant species makes these compounds important concerns of chemical taxonomy as well as important in the evolutionary responses of groups of herbivores and their food-plants. However, some secondary compounds have appeared in plants of distant phylogenetic relation, implying independent evolution and convergence on chemical defenses (Whittaker, 1970).

The majority of the toxic or repellent compounds fall within the following chemical groups (which can overlap due to the complexity of the structures): alkaloids, glycosides, phenols and tannins, organic acids, saponins, terpenes and steroids, and other essential oils. These represent various offshoots from the major metabolic pathways but have broadly similar origins with acetic acid and amino acids as the major starting materials. Extensive discussion of secondary plant substances and their metabolic pathways may be found in texts and monographs (Bonner and Varner, 1965; Harborne, 1964; Haslam, 1966; Pridham, 1967; Manske and Holmes, 1950-1968).

1) Phenols and Tannins

Plant phenolics are second in abundance only to carbohydrates. Substances of this large grouping are aromatic in the sense of having one or more benzene rings in their structure. The compounds are structurally diverse and include coumarins, quinones, lignins, flavonoids, and tannins; many occur as glycosidic derivatives. Antibiotic properties of plant phenolics are well known (Levin, 1971). They become localized around points of infection where they increase in concentration and undergo oxidation or hydrolysis to form more toxic derivatives. This action is probably important when herbivores damage plants and may be considered as a defense (Miles, 1969).

Perhaps the most effective and the most common defensive phenols are the tannins. These polymeric phenols are subdivided into two groups: "hydrolyzable" tannins are readily hydrolyzed by acids or enzymes into a sugar or related alcohol and a phenolcarboxylic acid; "condensed" tannins do not readily break down with acid but, may undergo progressive polymerization. Tannins have long been used in the tanning (hence the name "tannin") of leather because of their affinities for proteins such as collagen. Because of their economic importance, much literature exists on the chemistry and distribution of tannins in higher plants (Nierenstein, 1935; Bate-Smith, 1957; Haslam, 1966).

Feeny (1968) suggested the ecological importance of tannins by hypothesizing that the presence of increasing amounts of tannins in oak leaves may be one factor influencing the selection for early lepi-

dopteran larval feeding periods on oak leaves. Just as tannins can combine with proteinaceous collagens, tannins can bind to enzymes and other proteins in animal digestive tracts. Goldstein and Swain (1965) demonstrated that tannins inhibit enzyme activity and suggested that condensed tannins are more effective than hydrolysable tannins because they form more stable cross-linkings with proteins. Feeny and Bostock (1968) discovered that moth larvae stopped feeding at the same time that condensed tannins appeared in oak leaves. Further experiments showed that oak leaf tannins formed complexes with leaf proteins and also bound to animal digestive enzymes such as trypsin at the physiological pH of the larval moth midgut; these effects were more marked with condensed than with hydrolysable oak leaf tannins (Feeny, 1969). Thus, tannins exhibit two mechanisms which lower the nutritional value of the plant proteins to herbivores: 1) tannins can bind to the herbivore's digestive enzymes and thus inhibit activity in digestive processes, and 2) tannins can combine with the proteins from the food plant and render them unavailable to the herbivore's digestive processes. The non-specific nature of the interaction between tannins and proteins probably accounts for their effectiveness against herbivores. The generalized nature of the tannin-protein complexes with extensive hydrogen and covalent bonds (Haslam, 1966) implies that it would be difficult for herbivores to develop detoxification pathways such as those that are known for alkaloids and other specific poisonous chemicals in plants (Hodgson, Self, and Guthrie, 1965). Experiments in which oak leaf tannins were radioactively labelled

showed that they were retained inside the peritrophic membrane of the winter moth midgut (Feeny, 1970). Plants, in order to prevent autotoxicity, sequester tannins into vacuoles or attach them to sugars, rendering them unable to bind to proteins. When cellular damage occurs by herbivores, parasites, bacteria, etc., the tannins are released and become active.

How do herbivores adapt to the presence of tannins in their food in view of the lack of specific detoxification mechanisms? Feeny (1968) hypothesized that alteration of life histories can take place in which feeding periods minimize the contact with tannins such as was proposed for larval moths and mature oak leaves. Herbivores such as leaf miners feed on spongy parenchyma of leaves rather than in the palisade layer, where tannins are believed to concentrate. Since tannin-protein complexes are known to dissociate at extremely high pH, it is possible that alkaline guts in herbivores may represent an adaptation to release a higher proportion of protein for digestion (Feeny, 1969).

2) Alkaloids

Alkaloids have been found primarily in higher plants. They are nitrogenous bases often with a heterocyclic ring structure. Like tannins, they are implicated as agents responsible for the bitter taste or unpalatability of many plants and tissues. Many are well known as toxins and highly effective poisons (Manske and Holmes, 1968). Alkaloids known for their effects on man include nicotine, caffeine, quinine, morphine, codeine, mescaline and lysergic acid.

These probably represent repellents and toxins which have evolved in response as defenses to plant enemies. Nicotiana alkaloids (e.g. nicotine) have been commonly used as insecticides and have been shown to be toxic to insect larvae in nature (Thorsteinson, 1960). The beetle Leptinotarsa is poisoned by fresh leaves of Nicotiana as well as by the addition of alkaloids from solanaceous plants into its diet (Fraenkel, 1969). Demissin and Tomatin, both alkaloids from Solanaceae, inhibit the feeding responses of larvae and hence cause death by starvation. Larval feeding of the diamond-back moth Plutella maculipennis is similarly inhibited by high concentrations of alkaloids in the leaves of Solanaceae such as Lycopersicum (Thorsteinson, 1960). The entire coffee family Rubiaceae is protected from feeding of lepidopteran larvae by the presence of quinine in leaves. Highly specific repellants to herbivores are also present within plant families. Ehrlich and Raven (1967) have shown that larvae of the moth Euchelia jacobaceae can be fed on most species in the compositae genus Senecio except S. visconcus. This species produces a gummy compound which renders its leaves inedible. When the alkaloid substance is removed by dissolution in alcohol, the leaves are eaten by the moth; application of the compound to leaves of normally edible species in the genus renders them unpalatable.

Alkaloid content of plants can vary with time as was demonstrated for tannins in oak leaves. Ehrlich and Raven (1964) found that insects feeding on leaves of alkaloid-rich species of Papaver prefer young leaves in which concentrations of the secondary substance have

not yet built up. Thus feeding strategies of the herbivores can adapt to the chemical defenses of the plants.

As with tannins, plants often sequester alkaloids in vacuoles or attach them to sugars to form glycosides. These detoxification mechanisms in plants appear to be general to prevent autotoxicity from defensive chemicals.

Herbivore detoxification of plant alkaloids appears to be more common than detoxification of tannins. This is presumably due to the nature and the mode of action of the chemicals. Microsomal mixed-function oxidase enzymes in herbivores are effective in the detoxification of some, but not all, plant secondary compounds. Animal microsomal systems have an overall similarity from species to species; their main functions are breakdown of internal steroidal hormones and the degradation of foreign chemicals by enzymatic oxidation, reduction, or hydrolysis often followed by conjugation with other molecules (Williams, 1959; Schuster, 1964; La Du, Mandel, and Way, 1972). Tannins apparently are resistant to detoxification by microsomal systems because of their large and complex structures and their abilities to combine with proteins and inhibit enzymes. In contrast, alkaloids and various other toxic compounds are more easily rendered inactive by microsomal enzymes; species-specificity can be quite high. Thus, herbivores have the potential to be released from toxic effects of certain plant secondary compounds.

Chrysolina beetles apparently have evolved a mechanism for detoxification of hypericin, an alkaloid present in plants of the genus

Hypericum which prevents feeding by nearly all other herbivores. The result is that Chrysolina has a food supply unlimited by other herbivores and, in fact, has cued in on hypericin as an attractant. In other evolutionary "arms races" between plants and animals (Whittaker and Feeny, 1971) using chemical defenses and detoxification counter-attacks, the plant instead has won. Chrysanthemum cinerariaefolium produces pyrethrin which inhibits herbivory. Certain animals have evolved oxidase systems which can detoxify the compound. The plant, in turn, has elaborated other chemicals which inhibit the animal oxidase systems, allowing pyrethrin to be effective as a defense. Kreiger, Feeny and Wilkinson (1971) studied the microsomal oxidase systems of ten lepidopteran families, using the rate of epoxidation of aldrin to dieldrin as an index of enzymatic activity. Polyphagous species were found to have higher levels of activity than do monophagous species. The implication followed that the polyphagous species are adapted to detoxify a greater range of secondary compounds than are oligophagous species, and monophagous species are most limited in their ability to detoxify a range of compounds.

3) Terpenes and Steroids

Several interesting lines of defense are found among the terpenes and their many derivatives, including the steroids. Ecdysones (molting and metamorphosis hormones) and their analogs occur in plants and can disturb the endocrine control of insect life cycles (Kaplanis et al., 1967). Precise timing and concentrations of these compounds are necessary for normal animal growth and development. Plants, by

means of hormones, can kill their herbivores by either accelerating or preventing their metamorphosis. In Cecropia moths, metamorphosis can be fatally accelerated by 1 ppb. ponasterone (Williams, 1970).

Juvenile hormone (juvabione) present in balsam arrests the development of Pyrrhocoris at an immature stage (Slama and Williams, 1966). The importance of hormones as a means of herbivore control of plants is just beginning to be understood.

Sapogenins are plant steroids found in genera such as Yucca and Agave and are toxic to animals through hemolysis and other effects on membrane permeability. As with tannins and alkaloids, they occur as glycosides in plants to prevent autotoxicity. Cardiac glycosides consist of plant sterols linked to sugars; they are effective poisons which occur in the milkweed and dogbane families (Whittaker, 1970). Butterflies have evolved means to concentrate these antiherbivore compounds in their tissues and use them for defense against their predators (Brower and Brower, 1964).

Janzen, Juster, and Liener (1976) concluded that phytohemagglutinin in black beans (Phaseolus vulgaris) and other legumes is of adaptive significance in preventing attack by insects. Several hundred species of neotropical legumes are eaten by larvae of bruchid beetles. However, the bruchids are extremely species-specific; nearly all the species of bruchids in a given habitat cannot feed on any given species of potential prey. Callosobruchus maculatus feeds on cow peas (Vigna unguiculata) but not on nearby black beans. Black bean phytohemagglutinin added to the normal diet of C. maculatus in the labora-

