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Marine Invertebrate Chemical Defenses

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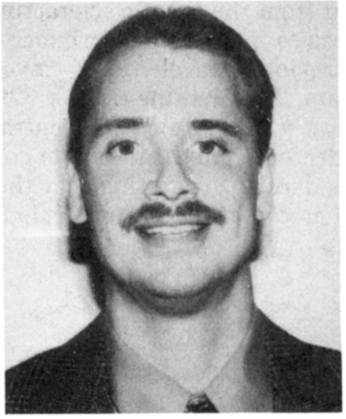
I. Introduction

Marine organisms have provided natural products chemists with a rich source of unusual secondary metabolites,[1] a point to which the other contributions to this issue will clearly attest. Yet, the biological functions of these compounds have only recently become the subject of study with the development of the interdisciplinary field of Marine Chemical Ecology. In this review, I will briefly explain the mechanisms by which marine invertebrates might use secondary metabolites for their protection and then summarize those studies for which there is ecologically relevant data on the ability of specific compounds to deter predation.

A. Types of Chemical Defense Mechanisms

Novel secondary metabolites that have been isolated from the tissues of marine invertebrates are most often assumed to have a defensive function. This perspective has undoubtedly arisen because of the "adaptationist" viewpoint that secondary metabolites must provide some service to the organisms that make them, inasmuch as they are often structurally complex or present in high concentrations, and therefore, metabolically expensive. Another reason, however, is the strong correlation between the absence of obvious physical defense mechanisms of an organism and the presence of unusual chemistry in its tissues: the richest sources of secondary metabolites have been soft-bodied sponges, cnidarians, sea slugs, and tunicates, while mobile (e.g., squid) and armored (e.g., crustaceans, snails, echinoderms) species generally lack secondary metabolites.

What are some of the defensive functions attributed to marine natural products? The most common is predator deterrence, but other suggested roles include prevention of fouling, inhibition of overgrowth, and protection from ultraviolet radiation. Most of the experimental evidence collected to date has addressed the antipredatory function, and the results of these studies are the primary subject of this review. Nevertheless, there are some compelling data that support some alternative defensive functions.



Joseph R. Pawlik received a B.S. degree in Biology from the University of Minnesota in 1982 and a Ph.D. in Marine Biology from Scripps Institution of Oceanography, University of California at San Diego, in 1988 under the direction of D. J. Faulkner. He began research on the chemical defenses of marine invertebrates in graduate school, but his dissertation research concerned the induction of marine invertebrate larval settlement by chemical cues. After a 2-year term as a Killam Memoral Postdoctoral Scholar at the University of Alberta, Edmonton (spent in residence at Friday Harbor Laboratories, University of Washington), he spent a year at Woods Hole Oceanographic Institution as a Science and Engineering Postdoctoral Fellow before moving to the University of North Carolina at Wilmington, where he is currently an Assistant Professor in the Department of Biological Sciences. His research interests include the influences of biological, chemical and physical factors on the settlement of planktonic marine invertebrate larvae, and the chemical defense mechanisms of soft-bodied, benthic marine invertebrates.

For benthic marine organisms, competition for space is generally believed to be intense, and the production of allelopathic metabolites is a suggested mechanism by which slower-growing species persist,[2] or established organisms deter the settlement of planktonic larvae.[3] Competitive interactions between sponges and scleractinian corals[4] and between soft (alcyonarian) corals and scleractinian corals[5] appear to be mediated by secondary metabolite exudation in mucus, which causes necrosis of the tissues of adjacent competitors. In the case of the coral-burrowing sponge *Siphonodictyon* sp., the mucus-borne metabolite siphonodictidine (**1**) increased respiration of the Pacific staghorn coral *Acropora formosa* when assayed at 0.01–0.1 ppm in seawater, with more dramatic toxic effects at higher concentrations.[6]

The inhibition of settlement (antifouling) by invertebrate-derived secondary metabolites has been re-

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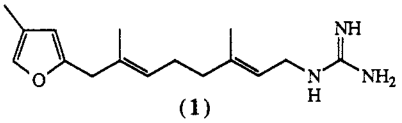
viewed recently.[7,8] Although there are several studies in which purified compounds from invertebrate sources have been demonstrated to have toxic effects on invertebrate larvae in laboratory assays,[9] it has not been established that secondary metabolites are released in a manner that would affect larval settlement under natural conditions of water flow.

Some secondary metabolites may act to shield invertebrates from ultraviolet radiation, an effect of particular advantage to sessile species that inhabit tropical coral reefs. A series of mycosporine-like amino acids isolated from the Pacific scleractinian staghorn coral *Acropora formosa* had absorbance maxima that spanned ultraviolet wavelengths: mycosporine-Gly (λ_{max} = 310 nm, **2**), palythine (λ_{max} = 320 nm, **3**), and palythinol (λ_{max} = 332 nm, **4**).[10] Concentrations of these compounds decreased in the tissues of corals collected at decreasing depths, suggesting that the compounds are produced in response to ultraviolet light exposure.[11]



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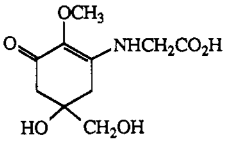

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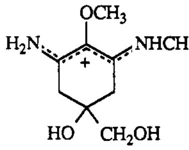

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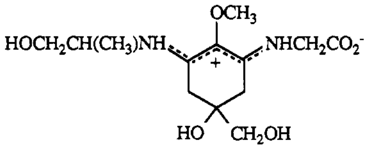


(4)

Despite the desires of both chemists and biologists to link the presence of unusual secondary compounds to a specific ecological purpose, the alternative hypothesis remains that these compounds have no function at all.[12] The process of natural selection does not generate perfect organisms that make optimal use of all their resources. It seems likely that some secondary metabolites represent so much "biochemical baggage": accumulations of side products of synthetic pathways, products of enzymatic activity on inappropriate substrates, or waste and detoxification products. Some metabolites may have played an ecological role in the distant past, (*e.g.*, as deterrents toward predators that have since gone extinct[13]) and are now produced because their synthesis is tied to that of a functional metabolite, or because selective pressures on the species are not sufficiently intense to result in the loss of the synthetic pathway. These "neutralist" alternative hypotheses are difficult to assess, however, because there is seemingly an endless number of "adaptationist" functions to which metabolite production can be assigned, including responses to generalist and specialist predators, competitors, or a range of physical adversities.

B. Experimental Evidence for Chemical Defenses

Although marine natural products chemists have been active in isolating novel secondary metabolites from marine invertebrates for over 3 decades,[1] most of the ecologically relevant studies of the natural functions of these compounds have been performed in the past few years.[8] Prior to that time, chemical defense was most frequently extrapolated from the toxicity of aqueous suspensions of crude organic extracts of invertebrates in assays using freshwater and brackish-water fish (usually goldfish, *Carassius auratus*, or mosquitofish, *Gambusia affinis*).[14] Recent evidence suggests that the toxicity of metabolites has little to do with the capacity of compounds to deter predators.[15] In a survey of the antipredatory chemical defenses of Caribbean sponges that is ongoing in my laboratory, we have found no correlation between the feeding-deterrent properties of sponge crude extracts and the published data on their toxicities.[16]

In light of the foregoing, the remainder of this review will focus on studies of antipredatory chemical defenses that have employed ecologically relevant assay techniques to investigate the deterrent effects of isolated and identified secondary metabolites. Not addressed herein is the large body of experimental literature, primarily biological, that provides evidence for antipredatory defenses on the basis of assays of crude extracts, but without subsequent isolation of active constituents,[17] and the even larger body of chemical literature that provides the structural elucidation of putative defensive metabolites without experimental evidence to support the claim.[18] This review will also not address chemical defense by association, which has been described for some species of small invertebrates that shelter themselves among the blades of chemically rich macroalgae, but do not themselves elaborate defensive metabolites.[19]

II. Antipredatory Chemical Defenses of Marine Invertebrates

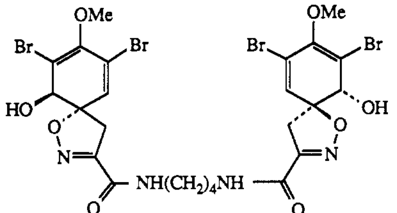
Some methods for assaying antifeedant properties of secondary metabolites from marine invertebrates are more rigorous than others. Minimally, extracts and purified compounds are incorporated into assay foods at natural concentrations and (along with the proper control foods) offered to predators that would be likely to encounter the organism in nature. When only small quantities of material are available, aquarium assays of small food pellets employing a single predatory species must suffice. Fish are the most commonly used predators in aquarium assays, because they respond quickly in feeding experiments and are important consumers in benthic environments. If larger quantities of metabolites can be acquired, field assays can be initiated in which foods are offered to a naturally occurring assemblage of potential predators (again, usually fish).

Extracts and compounds are usually incorporated into assay foods at concentrations expressed either as a function of dry weight or volume. For dry weight incorporation, a known weight of compound or extract is dissolved in a volatile solvent and applied to a known weight of dry food, followed by evaporation of the solvent. For volumetric incorporation, a known volume of organism is extracted, and the extract or compound isolated therefrom is homogeneously mixed into a food matrix (*e.g.*, carrageenan flavored with squid pureé) of the same volume. The latter methodology has the advantages of not requiring accurate weight measurements (important in field situations where balances may be unavailable), and perhaps of more accurately assessing the concentration of metabolites from the "watery" tissues of some sponges, corals, and tunicates.

A. Sponges

Of all invertebrates, sponges have yielded the largest number and greatest diversity of marine natural products. These common inhabitants of the benthos are excellent candidates for chemical defenses, inasmuch as they have a sessile lifestyle and soft, unprotected body tissues. Yet, for only a few species has the potential for chemical defense against predation been experimentally addressed. In many of these studies, investigations of sponge metabolites were initiated because the compounds were also found in dorid nudibranch molluscs, specialist predators of sponges that appear to have surmounted sponge defensive metabolites and often co-opt these compounds for their own use. Therefore, the reader is referred to the section on molluscan chemical defenses for further information on the activity of metabolites derived from sponges.

Secondary metabolites from four Southern California sponges were assayed for their ability to deter the feeding of a co-occurring predatory fish, the sculpin *Clinocottus analis*, as part of a survey of biologically active sponge compounds.[20] Both purified compounds and mixtures were assayed at 1.0% food pellet dry weight. A dibromotyrosine-derived metabolite present at 1.0% sponge dry weight, aerothionin (**5**), from *Aplysina fistularis* completely inhibited fish feeding, as did idiadione (**6**), a linear sesquiterpene present at 1.2% dry weight of *Leiosella idia*. Two sesquiterpenes from *Dysidea amblia*, pallescensin A (**7**) and furodysinin (**8**) were also complete feeding inhibitors, but both occurred at much lower concentrations in the sponge tissues (0.4% and 0.015%, respectively). Two other terpenoid compounds from the same sponge, ambliol A (**9**) and ambliofuran (**10**), were not deterrent, although they were present at 1.1% and 0.6% sponge dry weight, respectively. Mixtures of isonitriles (**11–14**) and their corresponding isothiocyanates (**15–18**) from *Axinella* sp. partially inhibited fish feeding; concentrations of the mixtures were 0.35 and 0.01% sponge dry weight, respectively.



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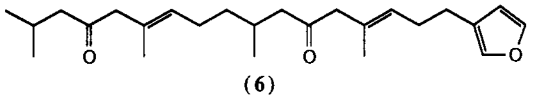

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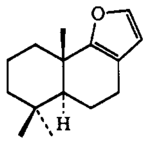

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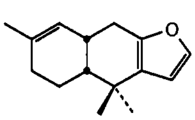

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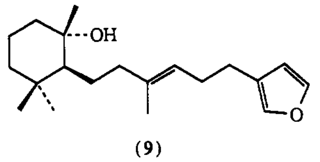

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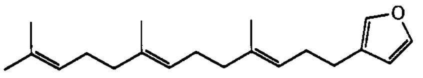

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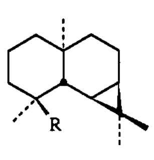

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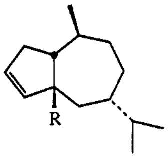
(15) R=NCS



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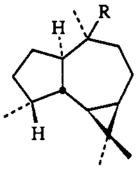

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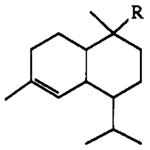
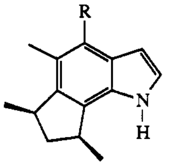
(17) R=NCS



(14) R=NC
(18) R=NCS

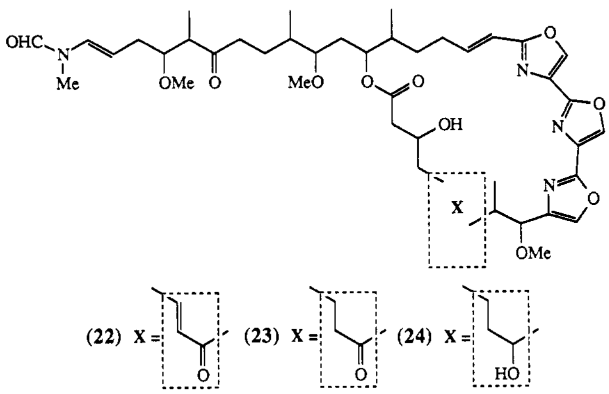
Another undescribed species of *Axinella* from western Australia yielded three polyalkylated indoles, herbindole A (**19**), B (**20**), and C (**21**).[21] The combined compounds, presumably at natural concentrations, deterred feeding of reef fishes in field assays conducted in Guam.



(19) R=CH_3
(20) R=CH_2CH_3
(21) R= 

Two undescribed species of the sponge *Halichondria* from the South Pacific contained macrolides that deterred predation by reef fish in aquarium assays.[22] The sponge metabolites were among a suite of macrolides (**22–26**) isolated from a nudibranch mollusc and its sponge prey. Halichondramide (**22**) and dihydrohalichondramide (**23**) were isolated from *Halichondria* sp. A from Kwajalein Atoll; the compounds were present at concentrations of 0.35% and 0.03% sponge dry weight. A second species, *Halichondria* sp. B, was collected in Palau and contained two related compounds, kabiramides B (**25**) and C (**26**) at 0.08% and 0.05% sponge dry weight, respectively. All four purified compounds were offered in food pellets to the Indo-Pacific reef fish, *Thalassoma lunare*, in aquarium assays. Each deterred feeding at 0.01–0.02% food pellet dry weight, making them the most potent feeding inhibitors reported to date.

Two sponge species from Guam, *Hyrtios erecta* and *Hyrtios altum*, were the subjects of a series of experiments investigating the defensive properties of their secondary metabolites.[23] Distinct chemotypes of *H. erecta* were found at two study sites; *H. erecta* A contained scalaradial (**27**, 2.4% dry weight), deoxy-



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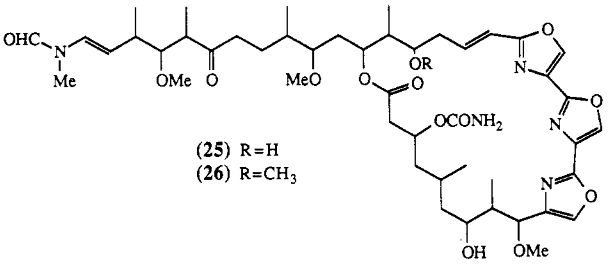
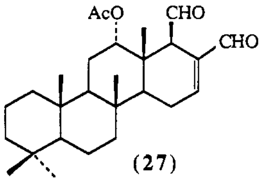


(25) R=H
(26) R=CH$_3$



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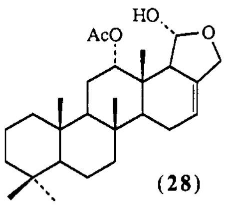

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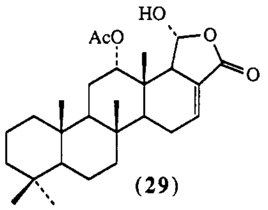

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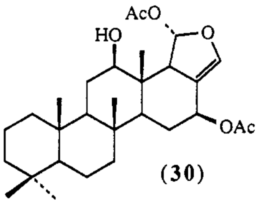
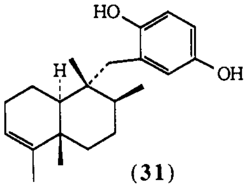


(30)

scalarin (**28**, concentration unknown), and scalarin (**29**, 2.0%), while *H. erecta* C contained heteronemin (**30**, 1.0%). *Hyrtios altum* also contained heteronemin at 1.8% sponge dry weight. Aquarium feeding assays were performed using the pufferfish *Canthigaster solandri*. Crude extracts of only one of the sponges, *H. erecta* A, deterred feeding of this fish at 7.0% dry weight, and none of the four compounds isolated from the sponges were deterrent, although one, scalarin, enhanced feeding at 1.0% dry weight. Field assays were also conducted in which pieces of food treated with extracts and purified compounds were offered to a natural assemblage of reef fishes. Results of these experiments were confusing: At one study site, extracts of *H. altum* and both chemotypes of *H. erecta* were deterrent when assayed at 7.0% dry weight on squid pieces, but when these extracts were incorporated into an artificial diet and assayed at the same location at 10.0% dry weight, they were not deterrent. At a second study site, extracts of *H. erecta* A assayed on squid pieces deterred feeding, while extracts of *H. erecta* C did not. At the first site, assays of squid treated with heteronemin deterred feeding at 1.0% dry weight, but scalaradial was not deterrent at 1.5% dry weight. In similar assays of all four metabolites incorporated into artificial diets and assayed at concentrations as high as 5.0% dry weight, none were deterrent. At the second study site, squid treated with heteronemin was deterrent at 1.5% but not 1.0% dry weight, and scalaradial was deterrent at 2.5% but not 1.5% dry weight. Interestingly, a 1:1 mixture of scalaradial and scalarin deterred fish feeding at 1.0% dry weight at this site.

In addition to the preceding compounds, the sponge metabolites avarol (**31**), latrunculin A (**32**), manoalide (**33**), secomanoalide (**34**), and a brominated diphenyl ether from a species of *Dysidea* (**35**) were assayed in the field on Guam reefs.[8] All the metabolites deterred the feeding of reef fishes when assayed at or below natural concentrations, except latrunculin A, which was deterrent only in aquarium assays against the pufferfish *Canthigaster solandri*.



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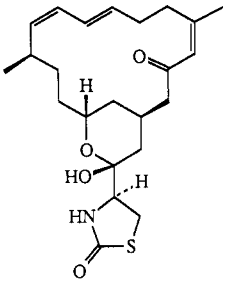

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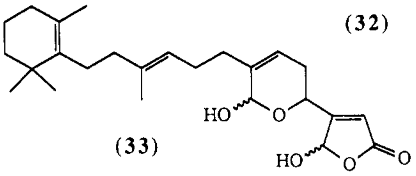

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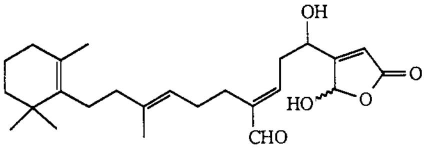

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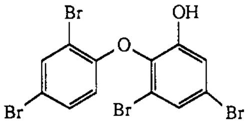

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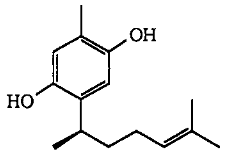
B. Cnidarians

Investigations of chemical antipredator defenses of cnidarians have been limited to species in two anthozoan orders, the Gorgonacea (sea whips and fans) and the Alcyonacea (soft and leather corals).[24] Unlike the hard corals (Scleractinia), members of these two groups have soft and fleshy tissues that, while often laden with calcareous spicules, appear physically undefended from potential predators.

Gorgonians are particularly abundant in the Caribbean, where their percentage coverage on coral reefs is often greater than that of hard corals.[25] A preliminary survey of 37 types of Caribbean gorgonians from at least 19 species and 11 genera was undertaken, employing a common reef fish, the bluehead wrasse *Thalassoma bifasciatum*, in ship-board aquarium assays.[26] Food pellets were treated with organic extracts of the gorgonians, and 5 treated and control pellets fed to a group of fish. Extract concentrations in the pellets were unknown relative to the concentrations in the gorgonian tissues at the time of the assay; concentrations were determined subsequently and varied widely relative to those in gorgonian tissues. Extracts of 19 types (51%) were highly unpalatable to fish (0 or 1 of 5 pellets eaten), four types (11%) were moderately unpalatable (2 or 3 of 5 eaten), and 14 types (38%) were palatable (4 or 5 of 5 eaten). Seven types were further assayed at decreasing concentrations of extract to determine the threshold of deterrence; most were deterrent at pellet concentrations near or below the concentrations that extracts occurred in the gorgonians. This preliminary survey served as the basis for further work on five species: *Pseudopterogorgia acerosa*, *Pseudopterogorgia rigida*, *Erythropodium caribaeorum*, *Pterogorgia anceps*, and *Plexaura homomalla*.

Crude extracts, purified compounds, and the calcitic spicules from the tissues of *Pseudopterogorgia acerosa* and *P. rigida* were assayed for defensive properties in both aquarium and field experiments.[27] Crude extracts of both species were deterrent in aquarium assays identical to those just described; moreover, when crude extracts were incorporated into carageenan food strips at volumetric concentrations ranging from 1.0% to 2.1% and deployed on the reef, the strips deterred feeding of the natural population of reef fish relative to control food strips. Purified compounds from both species were assayed further. Both curcuhydroquinone (**36**) and curcuquinone (**37**) from *P. rigida* deterred feeding in aquarium and field assays at concentrations below those present in the gorgonian tissues. A hydrocarbon mixture containing mainly curcumene (**38**) and the monoacetate of curcuhydroquinone did not deter feeding in either assay. It was suggested that pseudopterolide (**39**) functions as the defensive metabolite in the tissues of *P. acerosa*,[28] although this was not demonstrated experimentally. Calcitic spicules, which perfuse the soft tissue of these species, also deterred predation at concentrations of 34% and 68% by volume.



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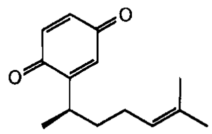

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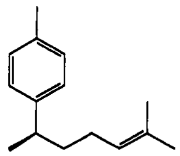

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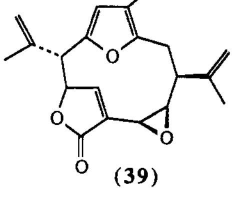

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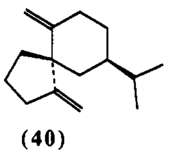
A crude extract of *Erythropodium caribaeorum* was strongly deterrent in preliminary aquarium assays,[26] inhibiting feeding at concentrations less than 1/10 of those present in the tissue of the gorgonian. Further experiments were conducted in the field.[29] A crude extract of *E. caribaeorum* also deterred feeding of a natural assemblage of reef fishes when assayed in carageenan strips at the same volumetric concentration as it occurred in the gorgonian tissue. Bioassay-directed fractionation of the crude extract localized the antifeedant activity to a fraction containing chlorinated diterpenoid erythrolides; a hydrocarbon fraction containing mainly erythrodiene (**40**) was not deterrent at natural concentrations. Further field assays of the purified compounds from the erythrolide fraction revealed that only erythrolide B (**41**) and erythrolide D (**42**) were unpalatable at natural concentrations, while erythrolide A (**43**) was not.



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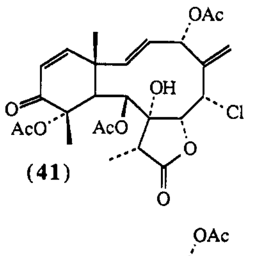

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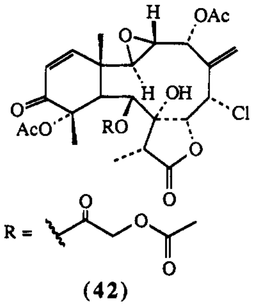
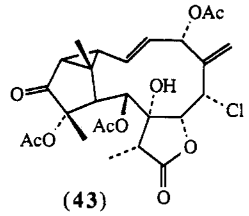
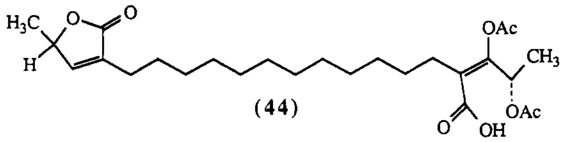


(42)



(43)

The antipredatory chemical defenses of *Pterogorgia anceps* were investigated with both aquarium and field assays.[30] A crude extract of the gorgonian deterred consumption of carageenan strips in field assays, as it had in previous preliminary assays.[26] Aquarium assays of fractions of the crude extract localized the deterrent activity to a fraction containing acetate-derived ancepsenolides (**44**–**46**); again, a less polar fraction containing hydrocarbons was not deterrent, although in this case the hydrocarbons were not identified. The mixture of ancepsenolides also deterred the feeding of a natural assemblage of reef fishes in a field experiment. Aquarium assays of compounds purified from the ancepsenolide fraction revealed that the deterrent activity could be assigned to a diacetoxy derivative of ancepsenolide **44**, but neither ancepsenolide (**45**) nor its monoacetoxy derivative **46** were deterrent when assayed alone or in combination.



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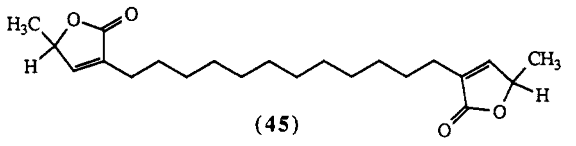

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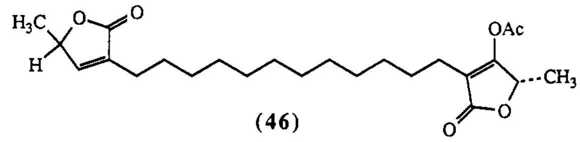

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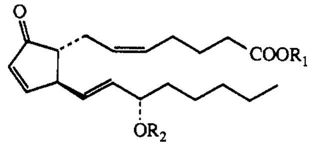


(47) $R_1 = H$, $R_2 = H$

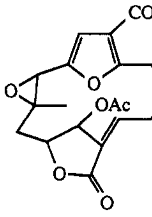
(48) $R_1 = CH_3$, $R_2 = COCH_3$

(49) $R_1 = CH_3$, $R_2 = H$

(50) $R_1 = H$, $R_2 = COCH_3$

In one of the first studies to use ecologically relevant experiments to investigate chemical defense, (15*R*)-prostaglandin A_2 (PGA_2) (**47**) was assayed in a study of the defensive role of secondary metabolites from *Plexaura homomalla*.[31] Food pellets treated with both (15*R*)-PGA_2 and the (15*R*) isomer (as hydroxy acids) were rejected by yellowhead wrasse fish, *Halichoeres garnoti*, in field assays. However, in a subsequent survey of gorgonian chemical defenses,[26] most gorgonians within the genus *Plexaura* yielded crude extracts that were palatable to another wrasse, *Thalassoma bifasciatum*. This inconsistancy resulted in a further study of the defensive properties of the prostaglandins in the tissues of *P. homomalla*.[32] The only prostaglandin derivatives found in the living tissues of *P. homomalla* are acetoxy methyl esters, with slow enzymatic hydrolysis after the gorgonian has been collected leading to the formation of acetoxy acids, hydroxy methyl esters and hydroxy acids.[33] Field assays of the acetoxy methyl ester of PGA_2 (**48**) at concentrations that were volumetrically equivalent to those present in the living gorgonian were not deterrent. In support of the previous study,[31] the hydroxy acid (**47**) was deterrent in field assays; in addition, the hydroxy methyl ester (**49**) and acetoxy acid (**50**) were also deterrent. Therefore, it appears that the prostaglandins present naturally in the tissues of *P. homomalla* do not offer an effective chemical defense against reef fish predation.

For soft corals (alcyonarians), evidence for chemical antipredator defenses come from one study of three species within the genus *Sinularia*.[34] In field assays against generalist fish carnivores conducted on patch reefs in Guam, crude extracts of *Sinularia maxima*, *S. polydactyla*, and an undescribed species of *Sinularia* deterred feeding at dry weight concentrations lower than those found in the tissues of the soft corals. An unnamed cembranoid diterpene **51** from *S. maxima* was also deterrent when assayed at natural concentrations. In the same study,[34] aquarium assays were conducted with the butterflyfish *Chaetodon unimaculatus* that preys specifically on coral tissues. Extracts of the three species of *Sinularia* were not deterrent toward *C. unimaculatus* at whole-colony concentrations, and in some cases, these concentrations enhanced feeding on food pellets over controls. Extracts were, however, deterrent at higher concentrations, and extract concentrations within the colony tips of *Sinularia* sp. fell below the deterrent threshold, while concentrations within the colony tips of *S. maxima* were deterrent. The cembranoid diterpene **51** was similarly deterrent toward *C. unimaculatus* at the concentration found in the tips of *S. maxima*. This result was corroborated by the feeding preferences of *C. unimaculatus* for *Sinularia* sp. over *S. maxima* in the field. The concentration of calcitic spicules in the soft coral tissues did not appear to play a defensive role for the coral specialist, *C. unimaculatus*, because the highest concentrations of spicules were found in the coral species most eaten by these fish; however, spicules reduced feeding of generalist fish predators in field assays.[35]



(51)

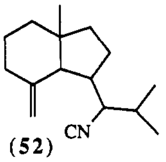
C. Molluscs

The phylum Mollusca has been the best studied in terms of invertebrate chemical defenses, with ecologically relevant data primarily for some species of gastropods. Molluscan chemical defense mechanisms have been the subject of an excellent recent review,[36] and the reader is directed there for a more detailed summary. In addition to organic compounds, several species of gastropods in the families Pleurobranchidae, Philinidae, Lamellariidae, and Cypraeidae secrete inorganic acids that appear to offer a chemical defense.[37]

The molluscan subclass Opisthobranchia includes several orders of shell-less sea slugs that have yielded a wealth of secondary metabolites, many of which are obtained by the molluscs from the organisms they eat. It has, in fact, been theorized that the evolutionary progression of shell loss in the subclass was concomitant with an elaboration of dietarily derived defensive metabolites.[38] Most of these slugs have specialized, often species-specific, diets: sponges for dorid nudibranchs (brightly colored slugs), algae for anaspideans (sea hares) and sacoglossans (green slugs).

Dorid nudibranchs prey primarily on sponges, often concentrating (and sometimes modifying) the sponge metabolites. As specialist predators, they appear to have evolved mechanisms for dealing with prey-produced compounds that would deter other predatory species and often store these comopunds in their brightly-colored mantle tissues. Investigations of antipredatory defenses of nudibranchs have often gone hand-in-hand with studies of the sponges they eat. The sponge diet of *Cadlina luteomarginata*, a nudibranch from the eastern Pacific, was determined by analyses of gut contents and the chemistry of slug extracts (see Sponges).[20] Assays performed with the intertidal sculpin fish, *Clinocottus analis*, indicated that idiadione (**6**) from *Leosella idia*, and pallescensin A (**7**) and furodysinin (**8**) derived from the sponge *Dysidea amblia*, were all deterrent at 1% dry weight of food pellet. Isonitriles (**11–14**) and the corresponding isothiocyanates (**15–18**) concentrated by *Cadlina luteomarginata* from the sponge *Axinella* sp. were only partially active in the assay.

Four Mediterranean nudibranchs were investigated for chemical defenses: *Phyllidia pulitzeri*, *Dendrodoris limbata*, *Glossodoris valenciennesi*, and *Glossodoris tricolor*.[39] Again, these dorid nudibranchs were found in association with sponges that contained similar secondary metabolites. Extracts of *Phyllidia pulitzeri* yielded isocyanosesquiterpenes and their corresponding formamides and isothiocyanates of the same composition as extracts of the sponge *Axinella cannabina*. One of these compounds, axisonitrile-1 (**52**), was assayed for both antifeedant properties and toxicity using the marine damselfish, *Chromis chromis*, and freshwater goldfish, *Carassius carassius*. Surprisingly, axisonitrile-1 was not an effective feeding deterrent, but was very toxic to fish at a minimum concentration of 8 ppm in seawater. The sesquiterpenoid dialdehyde polygodial (**53**) was isolated from the mantle tissue of *Dendrodoris limbata* and exhibited strong feeding deterrence at minimum concentrations of 30 $\mu g/cm^2$ of flake food.[39] Modification of polygodial with mild alkaline treatment to yield 9α-polygodial (**54**) resulted in a loss of the antifeedant properties. Extraction of *Glossodoris valenciennesi* yielded high concentrations of longifolin (**55**), which deterred feeding at 300 $\mu g/cm^2$ of flake food.[39] The accumulation of neither polygodial nor longifolin in these nudibranchs could be traced unambiguously to a sponge source, and polygodial was determined to be *de novo* synthesized by *Dendrodoris limbata*. Longifolin was subsequently found in vacuolar structures in the mantle tissues of the nudibranchs *Hypselodoris webbi*, *H. cantabrica*, and *H. villafranca* from European waters.[40] Both *Glossodoris tricolor* and its prey sponge *Cacospongia mollior* yielded the sesterterpenes scalaradial (**27**), furoscalarol (**56**), and deoxoscalarin (**28**), of which, the first was deterrent at 60 $\mu g/cm^2$, while the other two were active at 300 and 250 $\mu g/cm^2$, respectively.[39] Unlike the response to the dialdehyde polygodial (**53**), fish initially ingested flake food coated with scalaradial (**27**), but refused food for hours to days later.



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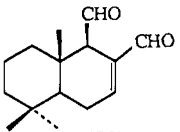
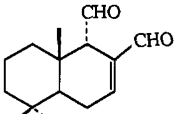


(53)



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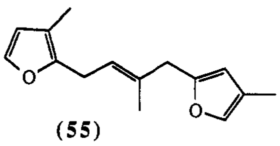
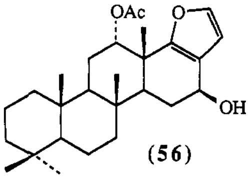
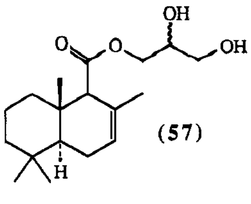


(55)

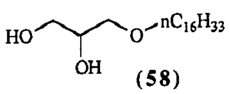


(56)

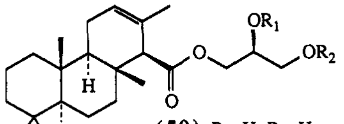
A cold-water nudibranch, *Archidoris montereyensis*, from British Columbia, was demonstrated to elaborate compounds in its dorsal mantle by *de novo* synthesis,[41] rather than by co-opting the compounds from dietary sources. Two of the metabolites isolated in the study, a dimenoic acid glyceride **57** and a glyceryl ether **58**, deterred feeding of the co-occurring sculpin fish *Oligocottus maculosus*, at concentrations of 18 μg/mg dry weight in aquarium assays. Three other compounds tested, a diterpenoic acid glyceride **59**, primary acetate **60**, and a farnesic acid glyceride **61**, were not deterrent at concentrations exceeding 100 μg/mg.



(57)

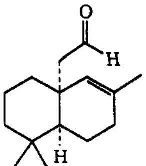


(58)



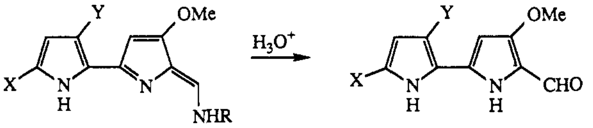
(59) R_1=H, R_2=H

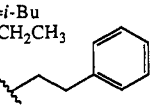
(60) R_1=H, R_2=Ac



(61)

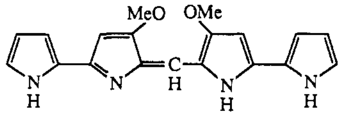
A series of bipyrrole compounds, the tambjamines **62–66**, act as chemical defenses for several nudibranch species, having been acquired from dietary sources in each case. In the Gulf of California, the nudibranchs *Tambja abdere* and *Tambja eliora* derive tambjamines A–D (**62–65**) from the bryozoan they eat, *Sessibugula translucens*.[42] In turn, the compounds are passed on to another nudibranch, *Roboastra tigris*, which feeds on the two *Tambja* species. The hydrolysis products **68–70** are formed during extraction of nudibranchs or bryozoans with methanol.[43] Mixtures of tambjamines **62–65** deterred feeding of the spotted kelpfish, *Gibbonsia elegans*, at concentrations ranging from 1–10 μg/mg food pellet dry weight, but the aldehydes **68–70** were not deterrent below 20 μg/mg. The chemical ecology of this system is quite complex: *Roboastra tigris* is sometimes deterred from eating *Tambja abdere* when the latter produces a quantity of mucus that contains tambjamines above a threshold of approximately 3 mg. *Tambja eliora* is able to locate its prey, *Sessibugula translucens*, by detection of low levels (10^{-10} M) of tambjamines in seawater. Thus, these compounds may deter generalist predators, but attract specialist predators.



(62) X=H, Y=H, R=H
(63) X=Br, Y=H, R=H
(64) X=H, Y=H, R=*i*-Bu
(65) X=H, Y=Br, R=*i*-Bu
(66) X=H, Y=H, R=CH_2CH_3
(67) X=H, Y=H, R= 

(68) X=H, Y=H
(69) X=Br, Y=H
(70) X=H, Y=Br

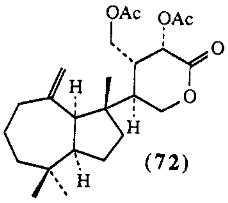
In the Indo-Pacific, at least three species of the nudibranch genus *Nembrotha* derive varying concentrations of tambjamines C (**64**), E (**66**), and F (**67**), from the ascidian *Sigillina signifera* (= *Atapozoa* sp.).[44] Extracts of the nudibranchs also contain tambjamine A (**62**) and a tetrapyrrole **71**, although these were not isolated from the ascidian. In field assays on two reefs on Guam, crude extracts of ascidians and nudibranchs, mixtures of tambjamines, purified tambjamine C and F, and the tetrapyrrole, all deterred fish feeding at or below natural concentrations. Tamjamines A and E were not deterrent at natural concentrations, but a 1:1 mixture of tambjamines E and F was deterrent when tested below natural concentrations.



(71)

Hexabranchus sanguineus, the large "spanish dancer" nudibranch common in the Indo-Pacific, contains oxazole macrolides that deterred feeding of a co-occurring reef fish, *Thalassoma lunare*, in aquarium assays (see Sponges).[22] The compounds were traced to a dietary source; in this case, sponges of the genus *Halichondria*. Five compounds were assayed in the study: halichondramide (**22**) was isolated only from the sponge, dihydrohalichondramide (**23**) and tetrahydrohalichondramide (**24**) were isolated from the nudibranch, and kabiramide B (**25**) and kabiramide C (**26**) were isolated from both sources. All four compounds deterred feeding at minimum concentrations of 0.01–0.02% dry weight, fully 1 order of magnitude lower than the concentrations present in the sponge and nudibranch tissues. Macrolides were concentrated in the dorsal mantle of the nudibranch and in the egg masses produced by the slugs.

Several spongian diterpenes isolated from the sponge *Aplysilla polyrhapis* were also isolated from the co-occurring nudibranch *Chromodoris norrisi* from the Gulf of California.[45] Shahamin C (**72**) and polyrhaphin C (**73**), found in both sponge and nudibranch, inhibited feeding of a co-occurring fish, *Thalassoma lucasanum*, in aquarium assays at 100 μg/mg dry weight of food pellet. A γ-lactone **74** isolated solely from the sponge exhibited the same activity. Four other compounds were not deterrent at this concentration: macfarlandin E (**75**), norrisolide (**76**), and polyrhaphin A (**77**) from both the sponge and nudibranch, and aplyviolene (**78**) from the sponge alone. Polyrhaphin C (**73**) and norrisolide (**76**) have also been isolated from *Chromodoris luteorosea* from the Cantabrian coast of Spain.[46]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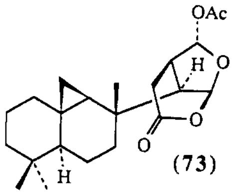
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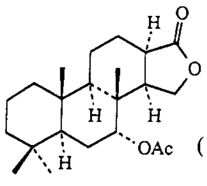
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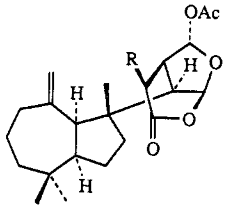
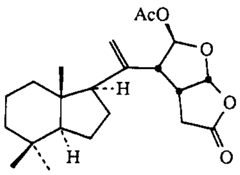

(75) R=OAc
(78) R=H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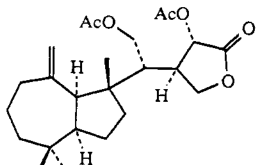
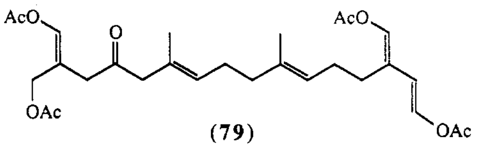

(77)

Three species of *Glossodoris* fed on two different chemotypes of the sponge *Hyrtios erecta* on Guam (see Sponges).[23] *Glossodoris pallida* preferred a chemotype containing scalaradial (**27**), while *G. hikeurensis* and *G. cincta* preferred a chemotype containing heteronemin (**30**); both compounds were sequestered by the nudibranchs. Assays conducted with purified compounds applied to pieces of squid and deployed in the field revealed that both compounds deterred natural fish predators at concentrations near those found in the nudibranch tissues.

Sacoglossans are opisthobranch slugs that feed on marine algae and sequester functional chloroplasts in the tissues of their mantle. Metabolites from three species of sacoglossans have been assessed for predator deterrence. The sacoglossans *Cyerce nigricans* and *Elysia* sp. from the Great Barrier Reef, Australia, feed on the green alga *Chlorodesmis fastigiata* and sequester the diterpenoid chlorodesmin (**79**).[47] Although whole sacoglossans of both species deterred feeding in a co-occurring reef fish, *Thalassoma lunare*, in aquarium assays, chlorodesmin did not deter feeding at 5% dry weight of food pellets. Chlorodesmin was, however, deterrent to reef fishes in field assays. Two additional pyrones **80** and **81** were isolated from extracts of *Cyerce nigricans*, but they were not effective feeding deterrents in aquarium assays.[48] The former compound **80** and related polypropionates were also isolated from the Mediterranean sacoglossan *Ercolania funerea*.[49]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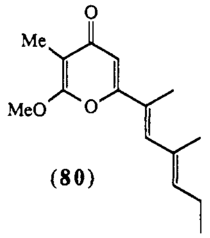
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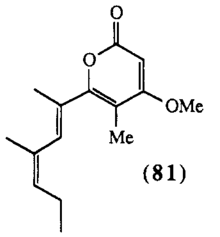
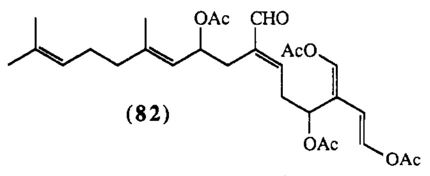
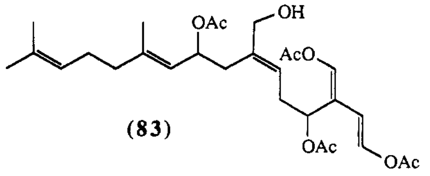
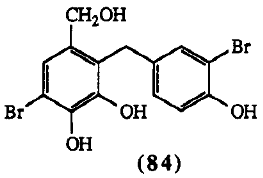

(81)

On Guam, the sacoglossan *Elysia halimedae* feeds on the green algae *Halimeda macroloba* and reduces the dietary algal metabolite halimedatetraacetate (**82**) to the diterpenoid **83**.[50] The diterpenoid **83** is secreted in mucus by the slug when disturbed, is present as 7% of the whole animal dry weight, and is present in the egg masses of the slugs. The diterpenoid **83** was an effective feeding deterrent in field assays at natural concentrations.


(82)

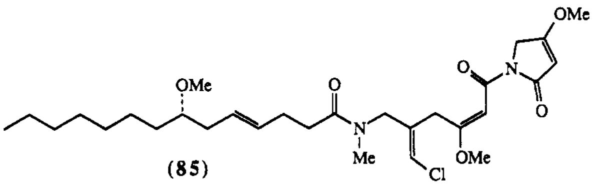

(83)

The Caribbean sacoglossan *Costasiella ocellifera* feeds on the green alga *Avrainvillea longicaulis* and sequesters the metabolite avrainvilleol (**84**) in its tissues.[51] Crude extracts of *Costasiella ocellifera* and avrainvilleol at 5% dry weight deterred feeding of the wrasse fish *Thalassoma bifasciatum*.

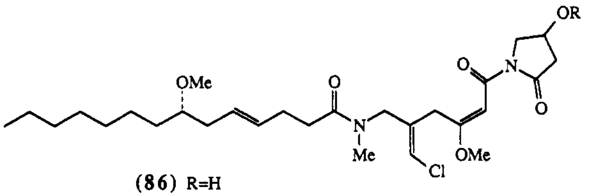


(84)

There is considerable anecdotal evidence for the defensive properties of the exudates of sea hares, but little experimental data.[36] The sea hare *Stylocheilus longicauda* feeds on the cyanobacterium (blue-green alga) *Microcoleus lyngbyaceus* on Guam and concentrates the metabolites malyngamide A (**85**) and B (**86**) from the algae, converting malyngamide B to its acetate 87.[52] Of the three compounds, only malyngamide B was deterrent at 2% dry weight in field assays; surprisingly, food pieces treated with the acetate of malyngamide B were preferentially eaten by predators over control pieces.

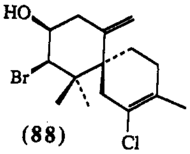


(85)

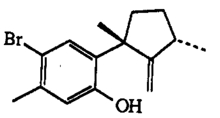


(86) R=H
(87) R=COCH$_3$

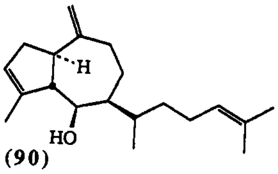
Other data on the deterrent properties of sea hare metabolites come from assays of algal natural products, inasmuch as many species of sea hares elaborate these dietary metabolites without modification. Field assays were performed to assess the deterrent properties of algal metabolites toward herbivorous fishes.[53] In these experiments, elatol (**88**), isolaurenterol (**89**), and pachydictyol A (**90**) were all feeding inhibitors, whereas aplysin (**91**), a compound commonly found in the digestive glands of *Aplysia* spp., was not deterrent.



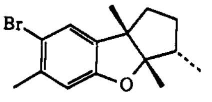
(88)



(89)

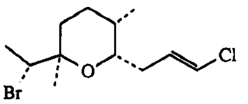


(90)



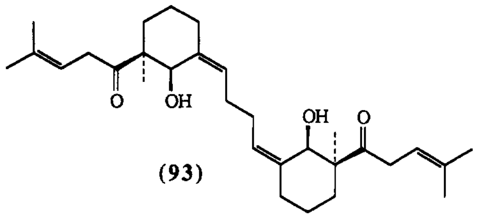
(91)

One species of cephalaspidean opisthobranch from Guam, the bubble snail *Haminoea cymbalum*, contains metabolites that have been tested for antifeedant properties.[54] Organic extracts contained kumepaloxane (**92**) at a concentration of 0.9% dry weight of the snails, and the compound was exuded in mucus when the animals were disturbed. Kumepaloxane applied to food strips at 1.8% dry weight deterred feeding of reef fishes in field assays and deterred the pufferfish, *Canthigaster solandri*, in aquarium assays.



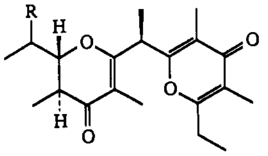
(92)

Prosobranch gastropods generally have sturdy shells, obviating any need for a chemical defense. A limpet from California, *Lottia* (= *Collisella*) *limatula*, appears to be an exception.[55] Intertidal fish and crabs readily ate pieces of the foot from four co-occurring limpet species, but rejected pieces from *Lottia limatula*. Feeding-deterrent activity was restricted to a single compound, the triterpene limatulone (**93**), which inhibited feeding of the kelpfish, *Gibbonisia elegans*, at minimum concentrations of 0.05% dry weight of food pellet. Limatulone may defend the intertidal limpet when foot tissues are exposed following nonfatal shell damage from wave borne rocks and debris.

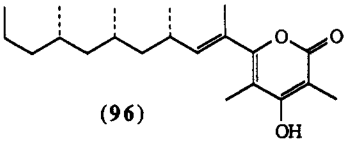


(93)

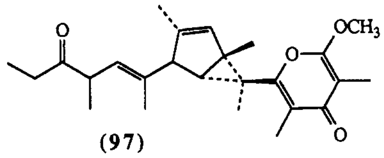
Pulmonate gastropods are largely terrestrial, but some species are marine. Siphonariid limpets produce a multitude of polypropionate compounds, of which only one, vallartanone B (**94**) from *Siphonaria maura* was



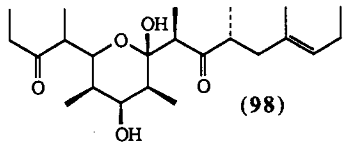
(94) R=H
(95) R=CH$_3$



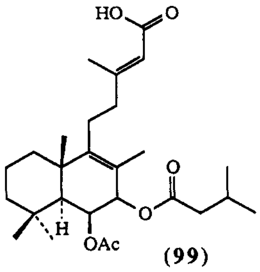
(96)



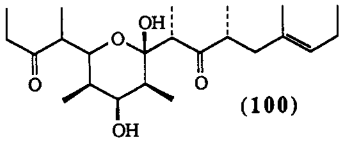
(97)



(98)



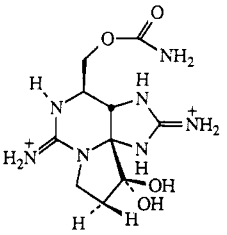
(99)



(100)

found to deter feeding of *Thalassoma lunare* at 100 μg/mg dry weight in aquarium assays.[56] Siphonariid metabolites that did not inhibit feeding in the same assay include vallartanone A (**95**), pectinatone (**96**), crispatene (**97**), denticulatins A (**98**) and B (**99**), and conicoic acid (**100**).[57]

Most bivalve molluscs are well protected by a pair of shells, and affording additional protection, heterodont bivalves (clams) generally bury themselves in sand or mud. Nevertheless, butter clams (*Saxidomus* spp.) appear to sequester paralytic shellfish poisoning toxins (PSPTs) from planktonic dinoflagellate sources as a chemical defense against vertebrate predators. *Saxidomus giganteus* and *S. nuttalli* from the eastern Pacific have nervous systems that exhibit tolerances to high concentrations of saxitoxin (**101**) derived from dinoflagellates such as *Protogonyaulax* spp., and these clams retain the toxins in their siphonal tissues over long periods of time.[58] Adult and juvenile glaucous-winged gulls, *Larus glaucescens*, and captive sea otters, *Enhydra lutris*, that were switched from being fed nontoxic butter clams to clams containing high concentrations of saxitoxin (445 μg/100 g clam tissue for gulls, 226 μg/100 g for otters) refused to eat either toxic or nontoxic butter clams (for gulls) or greatly reduced their prey capture and consumption rates (for otters).[59]



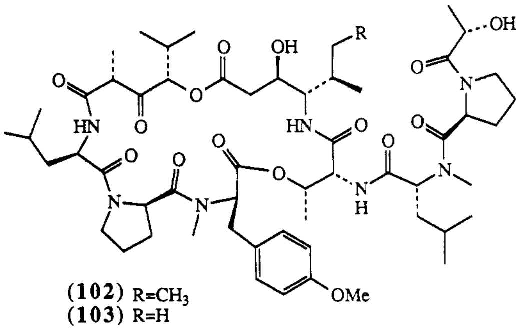
(**101**)

D. Ascidians

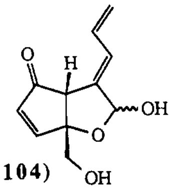
Like sponges, ascidians (sea squirts) are soft-bodied and sessile, with no obvious physical defense mechanisms. Many species of ascidians have highly acidic inclusions in their tunics (body coverings), in addition to high concentrations of heavy metals (e.g., vanadium). It was thought that these substances inhibited fouling and deterred predation,[60] but subsequent investigations have disputed this assessment.[61]

As discussed previously (see Molluscs), the tropical Pacific ascidian *Sigillina signifera* (= *Atapozoa* sp.) contains tambjamines C (**62**), E (**66**), and F (**67**), and tambjamine aldehyde (**68**).[44] Of these compounds, tambjamines C and F deterred feeding at or below natural concentrations in field assays conducted on Guam reefs, while tambjamine E was not deterrent.

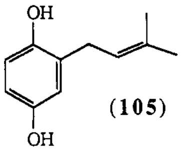
Ascidians produce tadpole larvae, which swim for a short time (less than an hour in most cases) before attaching to the substratum and metamorphosing into their adult form. Recently, a comparative study of the chemical defenses of adult and larval ascidians from several tropical and temperate localities was undertaken.[62] The Caribbean ascidian *Trididemnum solidum* and its tadpole larvae both contained a suite of didemnin and nordidemnin peptides; two of these, didemnin B (**102**) and nordidemnin B (**103**) were tested and both inhibited feeding of reef fishes in field assays at concentrations below those found in the ascidian tissues. A related ascidian from the same area, *Tridemnum* cf. *cyanophorum*, contained a mixture of didemnenones A and B (**104**), but these were not deterrent at natural concentrations. A prenyl hydroquinone **105** from the temperate Pacific ascidian *Aplidium californicum* did not deter feeding when assayed on Caribbean reefs at 0.25% dry weight. Patellamide C (**106**) and tambjamine E (**66**) from the Pacific tunicates *Lissoclinum patella* and *Sigillina signifera*, respectively, were assayed at natural concentrations on reefs in the Phillipines. Unlike previous assays on Guam, tambjamine E deterred feeding of reef fishes in these assays, while patellamide C was not significantly deterrent. An ascidian from the Gulf of California, *Polyandrocarpa* sp., contained a mixture of polyandrocarpidines A–D (**107**–**110**), which deterred feeding of the hermit crab *Clibanarius digueti* and the snails *Crassispira pluto* and *Tegula rugosa*.



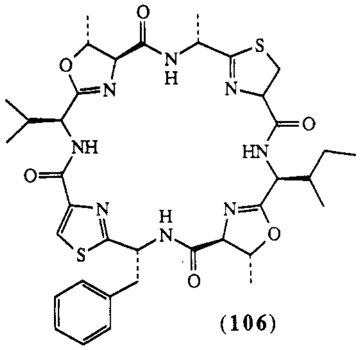
(**102**) R=CH_3
(**103**) R=H



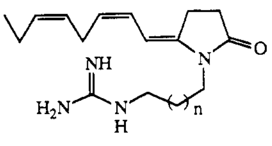
(**104**)



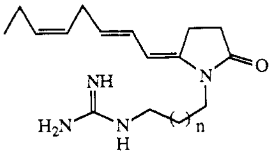
(**105**)



(**106**)



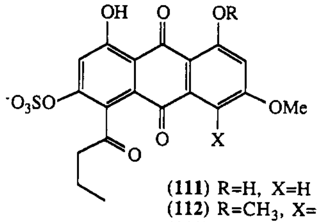
(**107**) n=3
(**109**) n=2



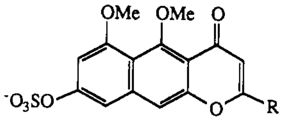
(**108**) n=3
(**110**) n=2

E. Other Invertebrates

Crinoids, or feather stars, are suspension-feeding echinoderms that are particularly abundant on Indo-Pacific coral reefs. Polyketide sulfates isolated from two Australian species, *Comantheria perplexa* and *Comatula pectinata*, were subjected to aquarium assays at natural concentrations.[63] A mixture of polyketide sulfates from *Comatula pectinata* (**111–113**) at 2% by volume deterred feeding of all the marine fish it was assayed against: *Selenotoca multifasciata, Monodactylus argenteus, Acanthopagrus australis, Platax orbicularis*, and *Trachurus mccullochi*. A mixture of compounds from *Comantheria perplexa* (**114** and **115**) at 0.2–2% by volume deterred feeding of the first two of the preceding fish species.



(**111**) R=H, X=H
(**112**) R=CH_3, X=H
(**113**) R=H, X=CH_3



(**114**) R=CH_3
(**115**) R=$CH_2CH_2CH_3$

III. Directions for Further Research

Investigations of the ecological roles of marine invertebrate secondary metabolites have only just begun, and the collaborative associations between natural products chemists and biologists will likely lead to continued growth of the field of Marine Chemical Ecology. Antifeedant assay-directed fractionation of organic extracts of invertebrates may lead to the isolation of new compounds that have been missed by more conventional isolation techniques. Moreover, a large body of biological literature implicates defensive metabolites in the ecology of various invertebrates, but the compounds are not yet known.[17] The chemical ecology of planktonic organisms is largely unstudied, although biological evidence suggests that phytoplankton,[64] jellyfish,[65] pelagic molluscs,[66] and crustacean larvae[67] may all defend themselves with secondary compounds.

A surprisingly large number of metabolites from marine invertebrates that have been subjected to relevant assays do not appear to play a role in antipredator defense. Identification of these compounds, which may serve their source organisms in other ways (or not at all), is important in understanding the relationships between chemical structure and biological response (the latter being, in this case, distastefulness). For example, most polypropionates (e.g., **95–100**) and hydrocarbons (e.g., **38**, **40**) that have been assayed to date do not deter feeding. Seemingly minor changes in the stereochemistry [polygodial (**53**) vs 9α-polygodial (**54**)], structure (erythrolide B (**41**) vs erythrolide A (**43**)], or functionality (tambjamine A (**62**) vs tambjamine aldehyde (**68**)] of deterrent compounds renders them inactive. It is too early to make any generalizations about structure–function relationships and chemical defense, but further research in this area is likely to yield information important to chemists and biologists alike.

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