Article

Chemistry and Chemical Ecology of some of the Common Opisthobranch Molluscs Found on the Shores of NE New Zealand

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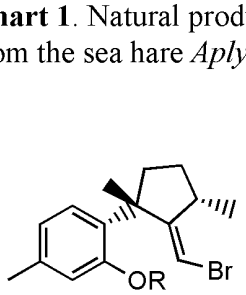
Opisthobranchs, sea hares and nudibranchs commonly known as sea slugs, are soft bodied marine invertebrates. Molluscs with a greatly reduced shell, the opisthobranchs, lack a physical defence mechanism and are potentially vulnerable to carnivorous predators. Loss of the shell in these animals is compensated for by several behavioural, anatomical, and physiological adaptations. Many opisthobranchs are cryptic and nocturnal, some can discard body parts when attacked, and some display an effective camouflage by sequestering pigments from their preferred foods. However, the most effective defence mechanism displayed by these organisms is a chemical one. Opisthobranchs are a rich source of cytotoxic, antimicrobial, antifungal, neurotoxic, and antimitotic natural products which have been shown to deter feeding by many natural predator species.[1] These defensive natural products are also known as secondary metabolites. Unlike the primary metabolites such as nucleic acids and proteins implicated in essential growth and differentiation processes, secondary metabolites are not directly involved in the basic maintenance functions of an organism. They do however play an important role in enhancing an organism's survival. Marine natural products have been assigned various ecological roles such as predator deterrence, mediation of competition, facilitation of reproduction, inhibition of overgrowth, protection from ultraviolet radiation, defense against pathogens and the induction of settlement.[2] Secondary metabolites are either diet-derived or made *de novo* by direct synthesis. Sea hares are herbivores that forage on red algae and sequester algal secondary metabolites into their own tissues. Nudibranchs can both sequester their prey's secondary metabolites and biosynthesise their own.

Over the past six years we have studied the secondary metabolite chemistry of several common local opisthobranch mollusc species; namely the sea hares *Aplysia parvula, A. dactylomela* and *Bursatella leachii*, and nudibranchs *Dendrodoris denisoni* and *Ceratosoma amoena*. In addition, by screening local species of algae we have identified possible dietary sources of these compounds and have established and quantified the anatomical distribution of secondary metabolites in selected organisms.

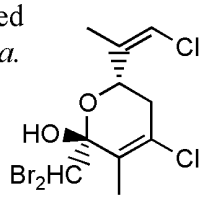
Natural product chemistry of sea hares

Sea hares are small, slow moving molluscs with a soft shell buried in the mantle tissue. They feed on micro and macro algae and display similar colouration patterns to their dietary plants. Most of the compounds isolated in our work on sea hares are either algal natural products or slightly modified derivatives. For example, the sea hare *Aplysia parvula* at the time of collection was feeding on the red alga *Plocamium costatum*. Costatone, the main metabolite in this sea hare is a known terpene natural product found both in an Australian collection and our own study of the alga *P. costatum* (Chart 1).[3] A comparison on the relative concentration of costatone between the sea hare and the alga, as quantified by ^{1}H NMR spectroscopy, revealed that costatone is fourteen times more concentrated in the sea hare (Fig. 1).

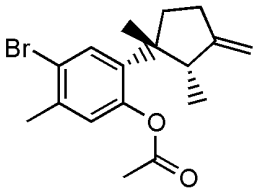
Chart 1. Natural products isolated from the sea hare *Aplysia parvula.*



Isolaurenisol, R=H
Isolaurenisol acetate, R=Ac



Costatone



Allolaurinterol acetate

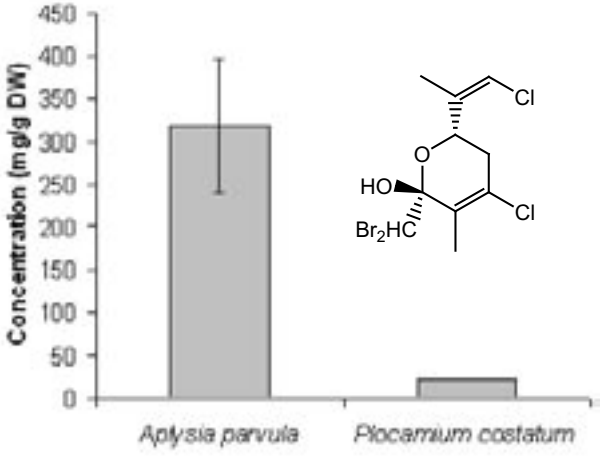
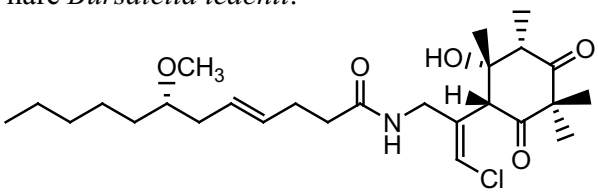


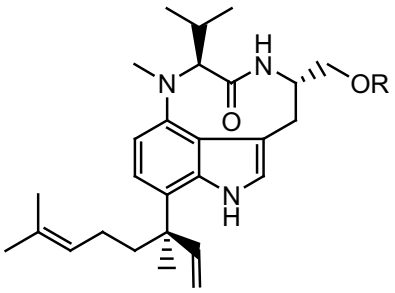
Fig. 1. Concentration of costatone in *Aplysia parvula* and its dietary plant *Plocamium costatum*; values quantified by ^{1}H NMR; data for *A. parvula* are presented as mean ± SD, n = 4.

Studies of the sea hare *Bursatella leachii* and its documented preferred food, the blue-green alga *Lyngbya majuscula*, yielded a new alkaloid malyngamide S as well as two other known natural products lyngbyatoxin A and its acetate (Chart 2).[4,5] Upon comparison of the sea hare and the dietary alga chemistry, compounds malyngamide S and lyngbyatoxin A were present in both organisms whereas lyngbyatoxin A acetate was never detected in the blue-green alga. Such acetylation adducts are common in opisthobranch natural product chemistry. Neither the allolaurinterol acetate or isolaurenisol acetate that we have found in *Aplysia parvula* and *A. dactylomela* are true algal metabolites (Chart 1). The free phenol compounds, allolaurinterol and isolaurenisol have been reported from local collections of red algae *Laurencia distichophylla* and *Hymenea variolosa*.[6] Acetylation of the phenol group is thought to occur in the sea hare, most likely as a detoxification mechanism or a by-product of the acidic digestive system environment.[7]

Chart 2. Natural products isolated from the sea hare *Bursatella leachii*.



Malyngamide S



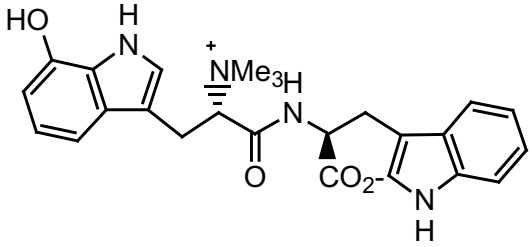
Lyngbyatoxin A, R=H

Lyngbyatoxin A acetate, R=Ac

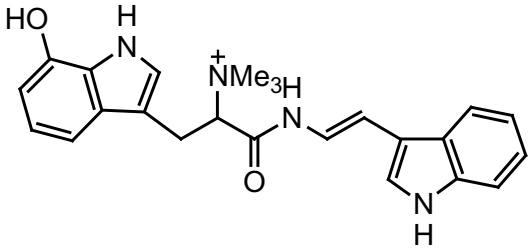
Aplysia dactylomela, apart from having known terpene metabolites allolaurinterol acetate and isolaurenisol acetate, was also the source of two new natural products dactylamides A and B (Chart 3).[8] Amino acid-derived metabolites have never previously been reported from the genus *Aplysia*. Dactylamides A and B were found in only a single specimen of *A. dactylomela* and it is yet to be resolved whether the sea hare acquires these tryptophan-derived dipeptides through diet or biosynthesis.

Our investigations have included both the sea hares and their dietary algae which were selected either because they were a previously documented dietary source or by our own field observations at the time of collection. We have shown that sea hares accumulate and concentrate selected algal metabolites in their own tissues. Gratifyingly, our secondary metabolite chemistry studies have found evidence of each sea hare dietary alga relationship established in previous ecological work.[5]

Chart 3. Natural products isolated from the sea hare *Aplysia dactylomela.*



Dactylamide A



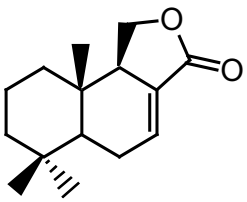
Dactylamide B

Natural product chemistry of nudibranchs

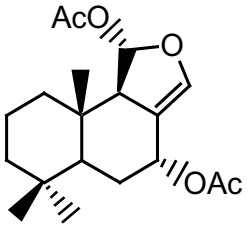
Nudibranchs are characterized by a complete loss of the shell. They are carnivores that prey on soft bodied invertebrates and sponges. As well as sequestering defensive metabolites from their prey, nudibranchs can also biosynthesize the same or very similar compounds themselves, a process known as de novo synthesis.[9] We have investigated the natural product chemistry of several local species: *Dendrodoris denisoni, Ceratosoma amoena, Tambja verconis* and *T. morosa*.

In the case of *Dendrodoris denisoni*, we have examined specimens collected from three different sites: Rabbit Island off the coast of the Coromandel Peninsula, Great Barrier Island and Matheson Bay. Cinnamolide, the major compound found in this species has previously been reported from a terrestrial plant but never a marine organism (Chart 4).[10] Interestingly, cinnamolide was detected in all the *D. denisoni* specimens that we have screened to date, independent of age or geographical location. The consistent occurrence of a compound in an organism throughout its range is considered an indicator of *de novo* synthesis.[11] During the isolation and purification work of the three minor *D. denisoni* metabolites, we found that olepupuane undergoes allylic methanolysis to give the methoxy acetal that decomposes into polygodial (Chart 4). All three compounds are common secondary metabolites of the genus *Dendrodoris* worldwide, and several species are able to biosynthesise olepupuane and polygodial when injected with the radiolabeled precursor, ^{14}C-mevalonic acid.[12] As very little is known about the ecology and the diet of this species and previous studies of the local ascidians and sponges have never found cinnamolide, olepupuane or related compounds, we suspect that *D. denisoni* might be synthesizing cinnamolide and possibly olepupuane de novo, rather then acquiring these compounds through diet.

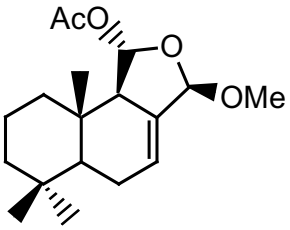
Chart 4. Natural products isolated from the nudibranch *Dendrodoris denisoni*.



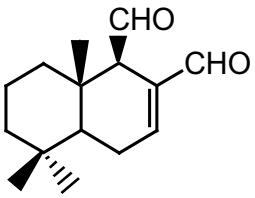
Cinnamolide



Olepupuane



Methoxy acetal



Polygodial

In contrast, another nudibranch species, *Ceratosoma amoena*, yielded an interesting ecological observation. At the time of collection the nudibranch was observed on the red alga *Hymenea variolosa*. We found a known red algal terpenoid secondary metabolite, allolaurinterol (Chart 1), in both the alga and the nudibranch. Nudibranchs are an exclusively carnivorous group of animals and do not have the digestive system physiology to break down algal tissues.[13] Therefore, *C. amoena* was not foraging on *H. variolosa* and sequestering its metabolites and an alternative explanation for the allolaurinterol in *C. amoena* might be that the nudibranch is preying upon some other organism that sequesters this compound from the alga. Locally, both *Aplysia dactyomela* and *A. parvula* are known to sequester allolaurinteriol, but since both sea hare species are significantly larger than *C. amoena* it seems highly unlikely that the nudibranch is preying on the two sea hares. However, many opisthobranch species are known to transfer their defensive metabolites into egg ribbons that they deposit on algae and other hard substrata. The nudibranch *C. amoena* may have foraged on chemically defended egg ribbons and thus sequestered allolaurinterol into its own tissues. At present this unusual trophic cascade seems the most likely pathway for the acquisition of a red algal metabolite in *C. amoena*.

The secondary metabolite chemistry of two other local nudibranch species, *Tambja verconis* and *T. morosa*, was studied as part of an ecological investigation of the genus *Tambja* carried out in collaboration with Haagh and Babcock at the Leigh Marine Lab.[14] *Tambja* nudibranchs are known for their ability to sequester a range of alkaloids, known as tambjamines, from their bryozoan prey *Bugula dentata*.[15] The present study found a range of related tambjamine metabolites in the two nudibranchs, but not all of the compounds were present in the bryozoan. Therefore, the two nudibranch species are not only sequestering secondary metabolites from their invertebrate prey but also structurally modifying these compounds and expanding their defensive arsenal.

Anatomical distribution of costatone and cinnamolide

Tissue localization of a secondary metabolite is an important ecological feature. Tissues which are directly exposed to predators, such as skin and mantle, should have a higher concentration of a defensive metabolite compared to the internal organs.[16] Two organisms, a sea hare *Aplysia parvula* and the nudibranch *Dendrodoris denisoni* and their major metabolites costatone and cinnamolide, respectively, were chosen for a metabolite localisation and quantification study. The digestive gland, a large enzyme-producing organ where digestion and absorption of food takes place, was taken as the internal organ, and skin and mantle as the external organs. The metabolites were quantified by ^{1}H NMR spectroscopy.

Costatone was found primarily in the digestive gland of the sea hare *A. parvula* (Fig. 2), but having close to 95% of the total metabolite content in an internal organ that is not in direct contact with a predator does not suggest a predator deterrence role for costatone. Concentrating algal secondary metabolites in the digestive gland might represent a simple detoxification mechanism by the sea hare. However, on many occasions during collection dives, we have observed that when large predatory fish such as snapper are offered live *A. parvula* initially they are taken in but then rapidly rejected, suggesting that there is a form of chemical deterrence associated with this organism. In contrast, *D. denisoni* stores cinnamolide both in the internal and external organs (Fig. 3). Our study showed that the relative concentration of cinnamolide in the external organs (skin and mantle) is significantly higher than that in the digestive gland consistent with previous investigations into secondary metabolite chemistry of the genus *Dendrodoris*. This strongly suggests that the organism utilises cinnamolide as a defensive, predator deterrent metabolite, but feeding deterrence studies with ecologically realistic predators need to be conducted in the field before any definite conclusions can be reached.

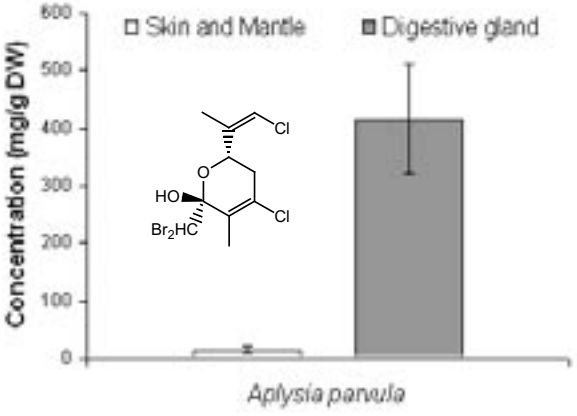


Fig. 2. Concentration of costatone in *Aplysia parvula* tissues; values quantified by ^{1}H NMR and presented as means ±SD, n=4.

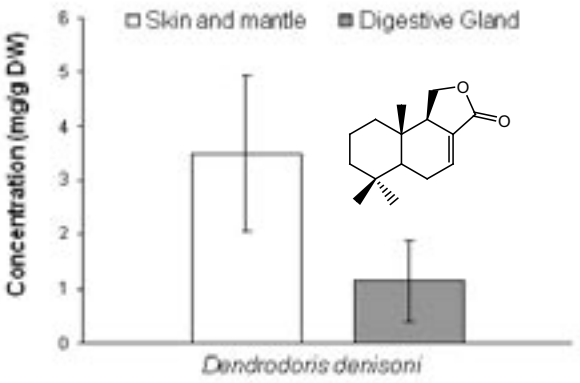


Fig. 3. Concentration of cinnamolide in *Dendrodoris denisoni* tissues; values quantified by ^{1}H NMR and presented as means ±SD, n=5.

Why study opisthobranch molluscs?

Chemical studies of opisthobranch molluscs provide a diverse source of new isoprenoid and amino acid-derived natural products. The evolution of chemical defences also represents an interesting ecological study of these organisms. It has been suggested that the shift from a hard-shell physical defence to a soft-bodied chemical one has been facilitated by a newly acquired ability to sequester, safely store and modify secondary metabolites by a hard-shelled ancestral species.[17] Sea hares display a diet-dependant acquisition of chemical defences. Their dietary algae are the primary source of their own secondary metabolites. Many studies have shown that purified sea hare secondary metabolites act as effective feeding deterrents, but their anatomical distribution suggests otherwise. It is not yet clear whether sea hares are a true example of chemically-defended organisms. On the other hand, nudibranchs display complex methods of secondary metabolite acquisition and their optimized anatomical distribution. Some, such as the genus *Tambja*, sequester the secondary metabolites of their prey, while *Dendrodoris* spp. nudibranchs are able to biosynthesize their own.

Our ongoing studies of soft bodied opisthobranchs have yielded several novel natural products including malyngamide S, and dactylamides A and B. Other compounds such as costatone, cinnamolide and isolaurenisol have been fully characterized for the first time. Our studies focus not only on finding new sources of bioactive natural products, but also on understanding the dietary relationship, anatomical distribution, and quantification of secondary metabolites, thus giving insight into the chemical ecology of selected species of New Zealand opisthobranch molluscs.

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