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Chemical Defense and the Evolution of Opisthobranch Gastropods

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Opisthobranch gastropods and their marine pulmonate relatives have tended to lose their shells as a consequence of being protected by chemical defense. Metabolites obtained from food have been modified and deployed adaptively. The animals have sometimes evolved the capacity to synthesize metabolites that were originally obtained from food. Some evidence suggests that this capacity has evolved beginning with an initial stage in which only the end product is synthesized, followed by a series of later innovations in which precursors of that end product are added working backward. There is a complex history of changes in what the animals eat and how they utilize metabolites defensively. When a change in feeding habits has deprived the animals of their original defensive metabolites, other compounds are often pressed into service. Among these are polypropionates, which are not biosynthesized by any other eukaryotes. The polypropionates probably exist at low concentration and have some other function in animals that do not use them defensively. There is rigorous and compelling experimental support for the biosynthesis of metabolites by the opisthobranchs themselves. An herbivorous common ancestor has given rise to many herbivorous lineages and to a wide variety of carnivores. Diversification has to some extent corresponded to the taxonomy of the food source, but the animals have often come to exploit unrelated food organisms that share the same metabolites or have a similar texture. The remarkable adaptive radiation of these animals can be explained as a result of their capacity to innovate in how they utilize their food sources and deal with secondary metabolites.

KEYWORDS: Adaptive radiation; Aposematism; Biosynthesis; Coevolution; Chemical defense; *Funktionswechsel; De novo* synthesis; Gastropoda; Marine; Natural products; Nudibranchia; Opisthobranchia; Phylogenetics; Polypropionates; Pulmonata; Repugnatorial glands; Review

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Dedication

To the memory of

D. John Faulkner

10 June 1942 – 23 November 2002

Chemical Defense and the Evolution of Opisthobranch Gastropods

Preface

This volume presents the results of many years of collaborative work. A great deal of the planning and writing was done during visits to the Istituto di Chimica Biomolecolare of the Consiglio Nazionale delle Ricerche in Pozzuoli (Napoli). Much of the original research herein discussed was done there and at its predecessor the Istituto per la Chimica di Molecole di Interesse Biologico in nearby Arco Felice (Trincone, 2002). We wish to express our deepest gratitude to our many colleagues, collaborators and employees, too numerous to enumerate here, though many of them are cited in the references. We would like, however, to give special thanks to those who have been particularly helpful in the preparation of the manuscript. Margherita Gavagnin, Angelo Fontana, and Ernesto Mollo read drafts of the manuscript and provided advice and assistance in the course of its preparation. Raffaele Turco did an outstanding job preparing illustrations and the atlas of metabolites. Ernesto Mollo and Guido Villani provided many of the illustrations.

At the California Academy of Sciences we have had the assistance and support of other colleagues, especially Terrence M. Gosliner and his students. He read drafts of the manuscript and provided expert advice on all aspects of the biology of opisthobranchs, especially their taxonomy. He also provided many of the photographs that we have used. Rebecca Johnson read a draft of the manuscript and made unpublished results available. William J. Bennetta's meticulous reading of the manuscript provided numerous improvements, Alan E. Leviton encouraged this project, advised us on the manuscript, and did most of the work of getting it set up in print and published. We are particularly grateful to the Academy, which has a long history of research and publication on opisthobranchs as well as the best collections of them in the world, for publishing the work.

It is our pleasant duty to acknowledge an International Short-Term Mobility Grant from the Consiglio Nazionale delle Ricerche in support of a visit to Pozzuoli.

Introduction

A slug, by definition, is a modified snail — one in which a shell is lacking or at least inconspicuous. The change from snail to slug can be seen in the animal's embryological development. The young slug can generally withdraw into a shell, but as the animal gets older the soft parts of the body become relatively larger and the shell relatively smaller. Often the shell is cast off when the animal is still quite young, but sometimes it remains as a kind of vestigial organ, no longer providing protection but perhaps providing some skeletal support. It tends to be grown over by tissue and may become completely internal. The reduction of the shell can be followed through geological time. Intermediate stages in the transition between snail and slug are also preserved in extant representatives of several distinct lineages. Therefore it has happened repeatedly, in separate lines of descent.

But why should something like that happen? After all, gastropods, whether they are snails or slugs, are slow-moving creatures that cannot easily outrun predators. Shells help to keep them from getting eaten. But that is not the only advantage to having a shell. It is a kind of skeleton, supporting the snail's body and providing sites for the attachment of muscles. It also helps to keep terrestrial snails from drying out. On the other hand, it gets in the way, slowing the animal down and hin-

dering its movements. And it costs something to make a shell as well as to haul it from place to place. It would seem that either the advantages of getting rid of the shell outweigh the disadvantages, or the slugs have found some way to compensate.

So far as avoiding predators goes, there is a fairly straightforward answer. Slugs have an unpleasant taste. As a general rule, the gastropods that are eaten by human beings have relatively well developed shells, whereas those without shells are left alone. This is true even of the air-breathing terrestrial snails and slugs (pulmonates). Not everybody likes escargot, but their shell-less relatives are repugnant even to gourmets. The recipe contest for "banana slugs" that is held annually in Sonoma County, California was always intended as a joke. As to marine gastropods, quite a variety are eaten by man, including abalone (*Haliotis*) and various limpets. Rumor has it that one species of seaslug is eaten in China and another in Alaska. Anecdotes about tropical sea-hares (see Anaspidea) are better documented. However, occasional references to seaslug fisheries in the popular press are uninformed with respect to elementary zoology. The animals in question are not gastropods at all. They are not even mollusks. Rather, they are sea-cucumbers, or holothurians, and, like sea-stars and sea-urchins, they are echinoderms, more closely related to chickens and cows than to snails.

Marine slugs, unlike their terrestrial relatives, are often very colorful. They may be strikingly beautiful, as one can readily see from the illustrations in this volume. Because they have no shells, their beauty is only apparent while they are still alive. Pickled specimens soon fade into colorless blobs. Consequently they are popular with photographers but not with amateur collectors, so that there is less of a conservation problem than there is with snails. Color patterns have a variety of functions in nature. Many birds have colorful sexual ornaments that are displayed during courtship. The color patterns of snails are not used that way, for the animals cannot perceive them. Many seem to be examples of warning coloration. The brilliant colors advertise the fact that the slug is not good to eat. Generally speaking, the slugs are not good to eat because of chemicals that are contained in their tissues. These chemicals are often obtained from food but sometimes the slugs make the chemicals themselves. We will go into such matters in considerable detail later, but for the moment let us dwell upon an exemplary scientific problem. Namely, did snails turn into slugs and then, because they were undefended, become distasteful? Or was it the other way round? In other words, did snails become distasteful and then, the shell being redundant, evolve into slugs?

That sounds like the problem of "Which came first, the chicken or the egg?" Fortunately, an evolutionary biologist can easily solve that kind of problem. There were eggs a billion years (plus or minus a few hundred million) before there were chickens. And in fact it was precisely this problem — which came first, chemical defense or the absence of a shell — that inspired much of the research that went into the present volume. Faulkner and Ghiselin (1983) showed that in several lineages of gastropods there are both shelled and shell-less forms with defensive chemicals. Consequently the chemical defense must have originated while the sea slugs' ancestors were still defended by shells. That kind of reasoning can be applied to quite a variety of similar problems. Natural products chemists and systematic zoologists are able to put their respective expertise together and thereby give new meaning to both disciplines. It is a kind of synergism, or, as bioeconomists put it, combination of labor.

One of our goals — indeed, one might even say the ultimate goal for all science — is to place the data of natural history within the context of an explanatory historical narrative. Such a view of what scientists ought to do is out of line with an old tradition in the philosophy of science. According to that view, science deals with the laws of nature and nothing whatsoever else; anything that refers to individuals in the broad, philosophical sense of particular material bodies or events is, by definition, something other than science. An historical science would be a contradic-

tion in terms. Defining "science" that way may seem rather odd, for it would mean that although physics is a science, astronomy is not. So too with geology, paleontology, anatomy, embryology, genomics, zoology, botany, bacteriology and anything else that might by any stretch of the imagination be considered a natural history discipline. That definition is arbitrary, subjective, and inappropriate to an understanding of science as it is pursued by real scientists in real laboratories. It seems far more realistic to define science in terms of the goals and methods that actually guide research. Science is about both the laws of nature and the particular things to which those laws apply. Although some sciences (the nomothetic ones) emphasize the laws, others (the idiographic ones) emphasize the particular things. For example, Physics is nomothetic, astronomy idiographic. But the distinction is misleading. Although some sciences emphasize the nomothetic aspect and others the idiographic aspect, the two are investigated together, often in the context of the same research program. An explanatory narrative puts the two aspects together by describing what has happened and explaining it in terms of laws of nature.

Toward the end of the eighteenth century such sciences as mineralogy, botany, and zoology were statically descriptive. Natural history was not conceived of as history in the sense of describing and explaining the natural order in terms of what has happened and why. But with the rise of geology and the introduction of evolutionary thinking (or something like it) there was a gradual shift from natural history to the history of nature (Lepenies, 1976). The real breakthrough came with Darwin's accomplishment. On the one hand, in *The Origin of Species*, he said: "Our classifications will come to be, so far as they can be so made, genealogies…." (Darwin, 1859:486). Groups of organisms are branches of phylogenetic trees, and much of natural order is the product of historical accident and circumstance. On the other hand, Darwin proposed the theory of natural selection, which provides the law-like basis for explaining diversity, replacing the assumptions about a supernatural order that had so long been presupposed. Evolutionary theory could now provide the basis for explanatory narratives in biology. The transformation of biology into an historical science occurred gradually, however, and its integration with natural products chemistry is a particularly enlightening example.

Chemistry is generally thought of as one of the nomothetic disciplines, but natural products chemistry is somewhat of an exception. It has traditionally been associated with botany and *materia medica*. As is common knowledge, not just medicines, but also poisons, flavorings, perfumes and many other chemicals that are in daily use are derived from plants, fungi, and microorganisms, and occasionally from animals. People began to use herbal remedies long before the rise of civilization, and the folk medicine of primitive tribes continues to yield valuable contributions to science. The roles that such materials play in the lives of the organisms that produce them, however, are not so widely appreciated. Not everybody who smokes, for example, realizes that nicotine is a natural insecticide. Even scientists were slow to understand such matters.

The chemicals to be discussed later on in this work have been investigated largely in the hope that they would provide "drugs from the sea," as can be seen from three books devoted to that subject (Freudenthal, ed., 1968; Fautin, ed., 1988; Fusetani, ed., 2000). In the first of these books the emphasis was largely on plants. The mollusks covered were mainly venomous ones, such as cone shells and *Octopus*, and bivalves with toxins taken up from small plants filtered out of the water. The opisthobranchs were scarcely mentioned, and although the sponges were beginning to receive attention, the nudibranchs as a source of sponge-derived metabolites were as yet unknown. The first metabolites of marine organisms to receive attention from medical researchers were alkaloids, as one might expect since some of them, such as the tetrodotoxin that occurs in the flesh of some fishes and in the tissues of bivalves, are exceedingly toxic and often fatal to human beings who ingest them. Tetrodotoxin and related alkaloids are the main exceptions to a general rule noted by

Daly (2004): bioreactive natural products that are found in the sea rarely occur in non-marine habitats and, conversely those from non-marine sources are rarely found in the sea.

Although classes of similar chemicals occur in both habitats, the structures are usually quite different. This rule may seem a bit less odd when we consider the taxonomic groups that are numerically dominant in the sea, in fresh water, and on land. The dominant terrestrial organisms that have been rich sources of bioactive chemicals, namely, vascular plants and insects, occur around the periphery of the sea, where they are replaced by algae and crustaceans. The main groups of marine animals that are sources of such compounds are ones that are restricted to the sea or have just a few representatives in fresh water: sponges, soft corals, tunicates, echinoderms, and opisthobranch gastropods. Tetrodotoxin and related compounds are largely associated with tropical to semitropical aquatic or semiaquatic vertebrates.

The great attention that was devoted to marine drugs began in the 1960s, and was well funded thanks in large measure to lavish spending by United States governmental agencies and also by Canada, Europe, and Japan. After several decades of concentrated effort only a few such drugs have become commercially successful. A synthetic peptide identical to one that occurs in snails of the genus *Conus* is now available as ziconotide (Prialt®) and used as a pain-killer. Quite a number of compounds, such as kahalalide F (**Atlas 652**), have been found to be active against tumors, but they are still undergoing clinical tests (Faircloth & Cuevas, 2006). The sophisticated approach now being applied involves synthesizing variants upon molecules that have been found to have promising biological activity and also understanding the chemical control of the relevant biological processes. The interested reader may wish to consult some of the following recent review articles: Newman and Cragg (2004); Newman, Cragg and Snader (2000, 2003); Paterson and Anderson (2005); Li and Vederas (2009); Molinski, Dalisay, Lievens and Saludes (2009).

Before he died at the age of sixty, D. John Faulkner published splendid annual reviews summarizing research on marine natural products chemistry in the journal *Natural Product Reports* (Faulkner, 1984a, 1984b, 1986, 1987, 1988, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000a, 2001b, 2002). These reviews are still exceedingly useful. The series has been continued by other authors (Blunt, Copp, Munro, Northcote & Prinsep, 2004, 2005, 2006; Blunt, Copp, Hu, Munro, Northcote & Prinsep, 2007, 2008, 2009). Other valuable review articles have also been published in the same journal and we refer to some of these in the text. A massive *Dictionary of Marine Natural Products* has been edited by Blunt and Munro (2008). The chemical literature presents something of a bibliographical barrier to the outsider because of such practices as the incomplete citation of references, leaving out titles and providing only the initial page. Herein the references are more nearly complete.

Early in the nineteenth century two unfortunate terms were introduced into the scientific literature: "organic chemistry" and "invertebrate zoology." Organic chemistry, introduced early in the nineteenth century by the Swedish chemist Jöns Jakob Berzelius, reflected the ancient doctrine of vitalism, according to which living organisms possessed a "vital force" that endowed them with capacities that transcended the laws of inert matter and could not be synthesized in the laboratory. The refutation of vitalism began with Friedrich Wöhler's synthesis of urea, performed in 1824 and made public in 1828. Subsequently the term "organic chemistry" was redefined so as to make it the chemistry of carbon compounds and the vitalism has become a dead metaphor. Yet as Hendrickson (1965) points out, during the early part of the nineteenth century organic chemistry was almost entirely concerned with the chemistry of natural products. Organic chemists continued to study them, but as a specialized branch of a larger discipline. The chemicals of interest to natural products chemists are mainly what are called "secondary metabolites" to distinguish them from the "primary metabolites," which are involved in the major biochemical pathways of the organism.

Secondary metabolites are secondary in the sense that they are made from the primary metabolites. Although secondary in that sense, they may be of crucial significance in the lives of the organisms in which they occur.

It was Jean-Baptiste Lamarck, the most influential pre-Darwinian evolutionist, who introduced the term "invertebrate" into the scientific literature. Invertebrates are animals without backbones. The sub-phylum of chordates to which we belong, Vertebrata, is a legitimate assemblage of animals all of which do in fact have backbones, and all of which are descended from a common ancestor. But invertebrates have nothing in common. They are just the animal kingdom minus one of its many branches. Dividing the animals in that way is like giving equal importance to British and non-British history. Lamarck believed that progress is a law, and thought that he could arrange all of the animals in a single series from lower to higher, culminating in Man. Simple animals, he thought, are continually being generated spontaneously and then giving rise to increasingly complex ones. He did suggest that adaptation to particular circumstances has led some animals to deviate somewhat from the general tendency for progressive change. But for him branching was unimportant. His classifications, because they arranged the materials in series, or "grades" proceeding from lower to higher, did not reflect the underlying pattern of diversification through time.

Darwin, on the other hand, recognized the importance of common ancestry and evolutionary divergence. Arranging the materials in terms of recency of common ancestry meant that classification could be truly historical. The different groups of organisms could now be seen as diversifying, and adapting to ever-changing circumstances. The groups would come to occupy an increasing variety of ecological niches, or, as he put it, "places in the economy of nature." The natural economy that he envisaged was a competitive one, in which the resources necessary for survival and reproduction were of crucial significance. The features of organisms could be explained as furthering the ability of their possessors to survive and reproduce—in other words, as adaptations in the sense of the products of an evolutionary process.

Darwin's theory of natural selection suggested that a much closer fit exists between organisms and their environments than had previously been realized. Impressive evidence for natural selection came in the form of discoveries about defensive adaptations that were made by three of Darwin's followers. Camouflage, which conceals an insect from a predator, has an obvious advantage in the struggle for existence, but what about the insects that are conspicuously colored? Darwin put that question to the co-discoverer of natural selection, Alfred Russel Wallace, who came up with the answer: warning coloration. The conspicuous animal is in effect advertising the fact that it is distasteful or otherwise not appropriate as food. Henry Walter Bates, who accompanied Wallace to South America, discovered what is called "Batesian mimicry" in his honor. Distasteful butterflies often live together with butterflies of other species that are not distasteful but look like them (respectively the model and the mimic). Bates was able to show that often the mimic and the model are quite distantly related, and that a butterfly species may mimic different models in different parts of its geographical ranges. Another of Darwin's early supporters, Fritz Müller, then a political refugee living in South America, discovered what is called "Müllerian mimicry," in which butterflies of several different species, all of them distasteful, resemble one another. The predators, mainly birds, are more apt to leave a butterfly alone if all the distasteful ones look the same.

Darwin (1862) himself wrote a book on orchids, in which he explained how these flowering plants reproduce with the aid of symbiotic insects. One of his points was that what might seem to be an unimportant structural detail often is of crucial importance in accomplishing fertilization. He also suggested that the structural arrangements are largely the result of historical accidents and are not the sort of thing that an intelligent creator would have produced. While he showed that natural

selection could account for even the most remarkable of adaptations, Darwin also reasoned that his theory would allow for a substantial amount of non-adaptive and even maladaptive change. Establishing the adaptive significance, or lack of it, of a feature may require a great deal of research. But adaptation has always been a contentious issue in evolutionary biology, and natural products chemistry has been a part of that discourse. Secondary metabolites have often been dismissed as useless byproducts that are excreted as waste.

A strong reaction against Darwinism set in toward the end of the nineteenth century, and the difficulty of understanding evolution was exacerbated by the problems of reconciling the theory of natural selection with the primitive sort of genetics that emerged around 1900. Various alternative views about evolution were popular in the early part of the twentieth century, and these included efforts to explain the features of organisms in terms of physical and chemical forces rather than adaptation by natural selection. Assertions that various features are non-adaptive were commonplace in a wide range of biological literature. Often such claims were made merely because the author could not imagine a use for something.

Objections to Darwin's theory on the basis of ideas about genetics collapsed in the 1930s. Theoretical population geneticists were able to show that selection is effective in producing change even when the advantage of one variant over another is quite small. Consequently biologists from various disciplines created the so-called "synthetic theory" of evolution during the period from around 1935 to 1950. It was very much like Darwin's original theory, but corrected with respect to matters of inheritance. Part of the synthesis involved taking a hard look at the supposedly maladaptive character of color patterns of butterflies and land-snails and re-interpreting them as adaptive features: often they were protective. Such efforts were really just beginning when, in 1959, the synthetic theorists celebrated their victory with the centennial of *The Origin of Species* (Tax, ed., 1960).

One point that Darwin had made, but which tended to be overlooked, or at least was neglected for some time, had to do with adaptation. Darwin conceived of adaptation by natural selection as resulting from differential reproductive success within species. The organism that has more descendants than its neighbor passes more of its traits to subsequent generations. Selection works through reproductive competition between individuals (not necessarily individual organisms as we shall see). That puts a severe restriction upon what evolutionary changes are possible. Yet if we understand that point, we are in a position to rule out certain kinds of explanations for the adaptive significance of one or another trait. It is not legitimate merely to suggest that a chemical that repels predators exists because it is "good for the species." If the predator kills the organism with a defensive chemical, the species will remain unchanged or indeed selection might favor its loss. In studying such matters one has to specify how natural selection has favored the evolution of the chemical in question. Sometimes the answer is that the individual that gets selected is a family rather than a single organism, but we defer our discussion of such matters until a later chapter.

Ruling out species-level or population level adaptation allowed for major advances in our understanding of adaptation in the 1960s and 1970s. But around 1975 something of a reaction sent in. This was in part linked to the controversy over "sociobiology" that emerged around that time. Some of the adaptive explanations that were being proposed were quite dubious, and open to criticism as having no more foundation than the products of imaginative literature. They were dismissed as "fairy tales" or "just-so stories." Such criticisms, however valid, were sometimes carried too far. Adaptive explanations in science are just like other hypotheses. They are no more than interesting possibilities until they have been tested by the appropriate methods. Such methods have been available ever since Darwin, and the evidence that is needed to apply them continues to accumulate.

The division of labor among scientific disciplines has many advantages, but the specialization that it involves is by no means an unmixed blessing. Often there is a considerable delay before scientists understand and appreciate developments outside of their own fields. The notion that secondary metabolites are non-adaptive features was still being taken seriously in the natural products literature into the 1980s, to judge from the fact that papers rebutting it were being published (Seigler, 1977; Jones, 1979; Ireland, Roll, Molinski, McKee, Zabriskie & Swersey, 1988). The ecological role of natural products has received an increasing amount of attention as their adaptive significance has gradually become more apparent. Ecological studies of secondary metabolites have been less concerned with evolution than one might expect. They have often addressed the question of adaptive significance, but the approach has tended to be an experimental one, and not the kind of comparative study that an evolutionary biologist would tend to favor. In general, experimental disciplines such as physiology and pharmacology have not focused much attention on evolutionary questions. We should point out, however, that among biochemists there has been a long tradition of addressing such topics as the origin and early evolution of life. Among the topics considered in that literature has been the evolution of biosynthesis. Although the origin of life is a topic for which few hard data are available, the evolution of biosynthesis can be studied by comparing living organisms. The biosynthesis of secondary compounds has been a major topic of interest to natural products chemists. One of our main reasons for writing this monograph has been to encourage them to study biosynthesis from an evolutionary point of view.

Systematic biology is the discipline (or group of disciplines) devoted to the study of biodiversity as such. It is largely concerned with classification and emphasizes the comparative approach. Although systematics was to some extent transformed into an historical science under the influence of Darwin, its traditions are much older, and the process of assimilating evolutionary thinking is still going on. The old naturalists were quite successful in arranging the materials of natural history in groups and providing catalogs and classification systems. Pre-Darwinian comparative anatomy provided detailed comparisons of the structure of plants and animals that were later given evolutionary interpretations. However, it was possible to continue comparing and classifying organisms as if nothing had really happened. Groups of organisms could be discovered simply by putting specimens together on the basis of their shared characteristics, without giving much thought to the historical processes that have been responsible for the similarities and differences. A morphological tradition that goes back to the German poet Johann Wolfgang von Goethe (1749-1832) sought to compare organisms on the basis of purely formal properties and abstract patterns, and to treat function as unimportant. That tradition remains influential.

Systematists, including botanists, zoologists, and bacteriologists, have often drawn upon secondary metabolites to supplement the usual anatomical or morphological features that are more readily seen and preserved in museum specimens. Often the goal has simply been to increase the number and variety of characters, especially in groups that have proved difficult to classify. We occasionally read of one metabolite or another being useful as a "taxonomic marker" or what a systematist would call a "diagnostic character." Only gradually, and recently, have such efforts been enhanced by consideration of what these chemicals do in the lives of the organisms, and of how the pattern of diversity has been produced by evolutionary processes.

The synthetic theory of evolution, which was considered essentially complete by the 1950s, largely, though not exclusively, focused upon those evolutionary processes that take place at the level of the species and the local population. Morphology and comparative anatomy contributed remarkably little at the time (Ghiselin, 1980, 2006). That was partly due to the fact that the architects of the theory were not particularly interested in them. For another thing, morphology had always de-emphasized function. And, finally, zoologists working with traditional materials and

approaches had little new to offer except, sometimes, speculations that filled in gaps in their data.

There was an opportunity to extend the synthesis by using new materials and an improved methodology. Molecular biology was particularly promising, especially when rapid sequencing techniques for proteins and nucleic acids became available. The new molecular techniques largely supplanted older ones such as immunology, so calling this a "molecular revolution" is a bit hyperbolic. What might be called a "restoration" rather than a "revolution" was the rediscovery of the importance of branching sequences in addressing problems of evolutionary history. There had been too much emphasis on arranging the organisms on the basis of how primitive and advanced they are and of how one group has given rise to another. The problem-solving situation called for placing the branches of a phylogenetic tree next to their closest relatives, and asking how the various lineages have diverged.

One of us (M.G.) did his doctoral research on the reproductive systems of opisthobranch gastropods, a group of mollusks that is closely related to the air-breathing snails and slugs called pulmonates. The change from snail to slug has occurred in many separate lineages of opisthobranchs and in several of pulmonates as well. "Parallelism" is the technical term for such an evolutionary pattern, in which several closely related lineages undergo the same basic modification. It is akin to what is called "convergence" in which initially different organisms become increasingly alike, usually because they occupy the same habitat or have the same way of life. Hummingbirds and hawkmoths provide a good example of convergent evolution. From a distance they look very much alike, but one does not have to be an expert to see that the resemblances are only superficial. Parallelism is much more difficult to recognize, for the resemblances, among slugs for example, can be quite detailed and extend to many parts of the body. Therefore parallel evolution poses an interesting methodological challenge. One solution to that problem is to find parts of the body that show divergence, rather than parallelism—in other words parts that become different instead of changing in the same basic way. The reproductive system evolves more or less independently of the rest of the body and is not much affected by the changes that are involved in transforming a snail into a slug.

The resulting phylogenetic tree left many questions open, but it did have some interesting implications. Among these was the fact that the various lineages of opisthobranchs tended to specialize in feeding upon a particular kind of food, or, what amounts to much the same thing, to having the same basic feeding mechanism. Gastropods in general move around quite slowly, but they are adept at finding, subduing, and processing "difficult" food items. The oral cavity usually contains a tongue-like strap called a radula, covered with hard teeth, which is very effective in cutting and tearing. Opisthobranchs tend to specialize on food items that other animals leave more or less alone. A fair number eat other opisthobranchs. Some eat macroscopic or filamentous algae. Many feed upon sponges. How that relates to natural products chemistry was not obvious until D. John Faulkner of the Scripps Institution of Oceanography entered the picture. He and his collaborators had been studying the natural products chemistry of many marine organisms, including opisthobranchs, and posed the "which came first" question that was discussed earlier in this introduction. We found evidence that supported his idea that use of defensive metabolites preceded the loss of the shell. Not only that, the use of chemical defense turned out to be even more widespread and various within the group than had been anticipated. We concluded that chemical defense has been the "driving force" behind the large-scale evolution of the group (Faulkner & Ghiselin, 1983).

Meanwhile, at Naples, where we would later join forces, G.C. had also become interested in the chemistry and biology of mollusks. He did his doctoral thesis on the black pigments called melanins of the cephalopod *Sepia officinalis*. Later he worked extensively on other marine animals including sponges. Toward the end of the 1980s he actively collaborated with Spanish biologists, notably the evolutionary ecologist Joandomenec Ros, and shifted his research program to opistho-

branch gastropods, especially to the biological role of the secondary metabolites that occur in them. There were obvious evolutionary implications. The data suggested stages, with accumulation of metabolites from the diet, followed by their transformation and finally by synthesis *de novo*. But the stages in question represented evolutionary grades, not the actual series of ancestors and descendants. What was needed was a detailed treatment of what had happened in different lineages. Upon getting together at Naples in 1995, we began to organize a collaborative research program. That collaboration benefited from the advice and assistance of colleagues at the California Academy of Sciences, which had become the leading center of research in the systematics of opisthobranchs thanks to the efforts of Terrence M. Gosliner and his group. We were thus in a position to combine the resources of state of the art laboratories in both natural products chemistry and opisthobranch systematics.

We decided that the results of G.C. and his collaborators' work could be recast in more strictly geneaological form and presented as review articles. For reasons of convenience we began with a discussion of one of the opisthobranch orders, Sacoglossa, a group for which there was ample material and for which a good classification was available (Cimino & Ghiselin, 1998). That was followed by a similar review of chemical defense and evolution in dorid nudibranchs that required more extended discussion (Cimino & Ghiselin, 1999). We then accepted an invitation to write a chapter entitled "Marine Natural Products Chemistry as an Evolutionary Narrative" in a book on marine chemical ecology (Cimino & Ghiselin, 2001). In that chapter we presented a somewhat broader survey of natural products chemistry and said something more about opisthobranchs but did not complete our survey of the group.

To some extent this monograph reiterates, updates, and completes what was said in our earlier publications. However, much of what is said here is new, and has not been reviewed elsewhere. We have taken the opportunity to present these materials in a way that makes them accessible to a somewhat broader range of readers, although this is not intended as a "popular" book. On the other hand the illustrations have been chosen with their aesthetic qualities in mind, and we hope that an even larger audience will enjoy them.

We begin with a survey of the main classes of metabolites that are of interest. Then we explain how they are synthesized and how they evolve. Then we discuss what happens to them in the bodies of the organisms. We consider the methods used, both experimentally and comparatively. Having completed such preliminaries we proceed to survey the groups of organisms that provide opisthobranchs with food and metabolites. Then we present a detailed evolutionary narrative, or scenario, for the evolution of chemical defense among opisthobranchs and some other gastropods. The scenario forms the bulk of the work. It is a bioeconomic scenario (Bertsch & Ghiselin, 1985). Bioeconomics is an emerging branch of knowledge that seeks to combine thinking like Charles Darwin with thinking like Adam Smith (Landa & Ghiselin, 1999). At the end we consider the implications of the scenario for our understanding of how life in general has diversified through time.

Chapter I

Biosynthesis and Biotransformation

Part 1: Biosynthesis

The ability of organisms to synthesize primary metabolites must have originated very early in the history of life. Indeed, we might go so far as to say that the origin of life and the origin of biosynthetic capacity are the same basic phenomenon. When a functional unit has arisen that is able to control and orchestrate a system of chemical reactions that sustain its existence and further its reproduction, we have what may reasonably be considered an organism. Much earlier, during what is considered prebiotic evolution, the precursors of such organized systems are supposed to have taken advantage of materials that were furnished by chemical reactions in the environment. The transition from the prebiotic to the biotic stage would then have involved the increasing ability to fabricate and transform a limited number of primary metabolites. Cells as we know them contain systems of biochemical pathways, such as the Krebs cycle, that make such metabolites available for the basic activities of the organism. Such activities are facilitated by enzymes, which are catalysts. Catalysts do not allow an organism to do anything contrary to the laws of nature. Instead they affect the rate at which reactions occur. In a biosynthetic pathway metabolites are modified step by step, with each step under enzymatic control.

It has been speculated that the earliest enzymes were RNA molecules. Like DNA, which is the hereditary material in most organisms, RNA can be replicated within the cell. But unlike DNA, RNA has also been shown to function as a catalyst or enzyme. According to the "RNA world" theory, DNA gradually replaced RNA as the hereditary material, and proteins took over most (but not quite all) of the enzymatic activity. So, at present, the main activity of RNA is that of transforming amino acids into polymers (including enzymes) using the hereditary material in the DNA as a template.

The evolution of biosynthetic pathways can occur by changes of the genes that control the structure of the enzymes. A mutation of a gene may alter the enzyme for which it codes such that it catalyzes another reaction and consequently gives rise to a different product. But that means losing the original product, which is not necessarily a good thing. New genes may also arise by duplication of a pre-existing one. Such gene duplication makes an identical enzyme available, which can then be modified, perhaps catalyzing reactions that did not occur previously. The pathway can thereby be changed by adding a new step at the end, or by giving rise to two different end products (Fischbach & Clardy, 2007). The history of the evolution of biosynthetic pathways can be reconstructed as a series of duplications and subsequent divergent modifications of the molecules and the genes that code for them. However, that is not the only thing that goes on, and we must avoid oversimplifying. In addition to genes that code for proteins, there are regulatory genes that affect what product is produced when, and where, and in what quantity. And the activities of cells and organs also come into play.

The opportunistic character of evolutionary change in general provides an important basis for reconstructing evolutionary history. New features arise from the modification of pre-existing ones. Darwin's follower Anton Dohrn (1840-1909) created the magnificent marine laboratory at Naples for the study of evolution among marine organisms. He also propounded what he called the principle of the succession of functions (Dohrn, 1875; translation in Ghiselin, 1994). According to Dohrn, new organs arise from earlier ones in an orderly and gradual sequence. A part begins having an initial main function, then it comes to have both the main function and a minor, additional one. With the passage of time the new function becomes increasingly important, finally replacing

the original one, and the organ as a whole becomes substantially modified in adaptation to the new function. Evolution by gene duplication is just a variation on that theme. In any case, research aims at establishing plausible precursors for modified structures.

Biochemists, particularly those who are interested in biosynthetic pathways, have applied other evolutionary principles. One example is the so-called "principle of recapitulation," which was popularized and elaborated by Darwin's follower Ernst Haeckel (1834-1919). Actually Haeckel derived the principle from Fritz Müller, who in turn was following Darwin's reinterpretation of pre-evolutionary concepts, but the details need not concern us here (see Ghiselin, 1997; Breidbach & Ghiselin, 2007). The simplistic version, in which the fine print is left out, tells us that ontogeny recapitulates phylogeny: in other words that as embryos develop they pass through a series of stages that repeat (recapitulate) the structures of their ancestors, beginning with a unicellular egg or zygote that is equivalent to a protozoan. Indeed, both Morowitz (1992) and Lahav (1999) treat the successive steps in biosynthetic pathways as exemplifying recapitulation at a molecular level. In many, perhaps most, cases, this interpretation is evidently correct and the reasons why are straightforward and clear. Suppose that there has been an evolutionary sequence in which the biosynthetic pathway has become increasingly lengthy by addition of a part at the end only. So, using a series of letters to represent the stages, we start with A→B, then go to A→B→C, then to A→B→C→D, and finally to A→B→C→D→E. Examples of such situations are available from the natural products literature. In some cases it is obvious that the intermediates, which are often present at low levels in the longer pathways, correspond to terminal products in ancestral organisms. The opisthobranch *Scaphander lignarius* contains a series of unique oxygenated arylalkenyl compounds (**Atlas 71-77**) and also, in minor amounts, a smaller ω-phenylpentadienal (**Atlas 78**) which may be its precursor in biosynthesis and perhaps in phylogeny (Della Sala, Cutignano, Fontana, Spinella, Calabrese, Domenech Coll, d'Ippolito, Della Monica & Cimino, 2007). (See the chapter on Cephalaspidea for a discussion of these lignarenones.)

Ordinarily the pathways can only be modified in certain respects. It is hard to imagine a case in which A→B→C→D could go to A→C→B→D, although perhaps D could drop out or B come from some other source. Evolutionary embryologists including Fritz Müller (1864), Aleksy Nikoliaevich Sewertzoff (1931) and later authors have stressed the point that recapitulation only applies to cases in which evolution has occurred by terminal addition. In some materials, terminal addition occurs, but it seems to be more the exception than the rule. What about biosynthesis? Is it possible to go, for example, from C→D to B→C→D to A→B→C→D? Here the stricture that changes must occur through stepwise changes applies, but the additions would be at the beginning, not the end. Our data suggested that something like this might happen in opisthobranchs, and we proposed that there can be evolution in the "retrosynthetic mode" in addition to the usual "anasynthetic mode" (Cimino & Ghiselin, 1999). Many years earlier Norman Horowitz (1945, 1965) had invoked evolution in the retrosynthetic mode in the context of a theory of the early evolution of life. In the hypothetical "soup" in which life is supposed to have evolved there would have been all sorts of molecules available in the environment and the organisms or pre-organisms would have used chemicals that resulted from a series of transformations that resulted from non-biotic activities. However, they would benefit from anything that increased the yield of such reactions, and genes giving rise to enzymes that catalyzed the final step would be favored by natural selection. New enzymes could then be added in the same way and for the same reason, working backwards.

We were led to propose such a mechanism for opisthobranchs from having found evolutionary sequences in which the slugs began to derive metabolites from their food and to use them defensively, often in modified form (Cimino & Ghiselin, 1999). Later, however, they became able to synthesize similar metabolites themselves (*de novo*). The possibility then came to mind that a

given pathway might evolve beginning with an ability to modify a metabolite from the environment somewhat, perhaps to make it more or less toxic. Once an enzyme with that effect had become available, evolution by gene duplication could occur, working backward to establish an entire pathway. Biotransformation would precede biosynthesis. In such cases, ontogeny definitely would not recapitulate phylogeny. Whether that is what really happened remains an open question, but some of our research has been inspired by that possibility. There are other alternatives, such as lateral gene transfer, that also need to be considered. We will come back to such issues in later sections of this essay.

This monograph uses a comparative approach to the diversity of both the animals and the metabolites, utilizing the systems of classification that have been worked out by biologists and chemists respectively. The text is organized so as to follow the biological system and tell an historical story, using some illustrations of the organisms and diagrams of their relationships. There is also a chemical classification, which has a different rationale and different rules of nomenclature. To facilitate comparison of the metabolites, we have presented structural diagrams of them classified on a chemical basis. This arrangement is presented as Appendix I. This "atlas" allows one to see the relevant similarities and differences among the metabolites. Another goal of this atlas has been to provide an inventory of the metabolites that are known from opisthobranch gastropods and their close relatives. We decided not to include a diagram of every known metabolite. Some of these are minor structural variants. Others are substances taken up with the food and not particularly interesting from the point of view of the biology of the animals. In both cases a few examples should suffice. Using the IUPAC rules of nomenclature, any organic compound can be given a name that describes the structure of the molecule, and occasionally we have given these. Of course the diagrams are much easier to use. Furthermore such names can be quite awkward. That helps to explain why kinds of molecules are given names that may be rather arbitrary and not very informative. Such names are often based upon the genus or species of the organism in which they were discovered. Some indication of the chemical structure is often given by means of suffixes, such as *-ol* for an alcohol or *-al* for an aldehyde.

Secondary metabolites are biosynthesized from primary metabolites. The vast majority of secondary metabolites derive from just a few classes of simple starting materials. Likewise the mechanisms through which the molecules get modified are not very diverse. Therefore we can provide an elementary, or introductory, treatment without having to oversimplify the subject. For somewhat more advanced accounts that are intelligible to a broad audience, we recommend the textbooks of Hendrickson (1965), Mann (1994) and Dewick (1997). Our understanding of metabolism can be improved by greater emphasis on the bioeconomic point of view. The thermodynamic efficiency of metabolism has often been discussed, but that is only a beginning. Having a flexible system of interchangeable parts is an important feature of well organized systems of production. Modular organization, with a few basic components giving rise to a diversity of products, simplifies the production process and allows it to be more effectively controlled.

Primary metabolism allows the organism to modify molecules and thereby make energy and materials available in ways that sustain life. The scheme shown in Figure I shows some of the main pathways that are involved in primary metabolism and how the primary metabolites provide materials that are used in constructing secondary metabolites. The anaerobic part, or glycolysis cycle, allows for the derivation of energy-rich compounds, such as ATP, from sugars and other chemicals either from photosynthesis or from food. The Krebs cycle makes use of oxygen and allows more energy to be derived. A pentose cycle is present in photosynthetic (more generally autotrophic) organisms only. It is absent in animals, and metabolites that derive from it must be obtained from food.

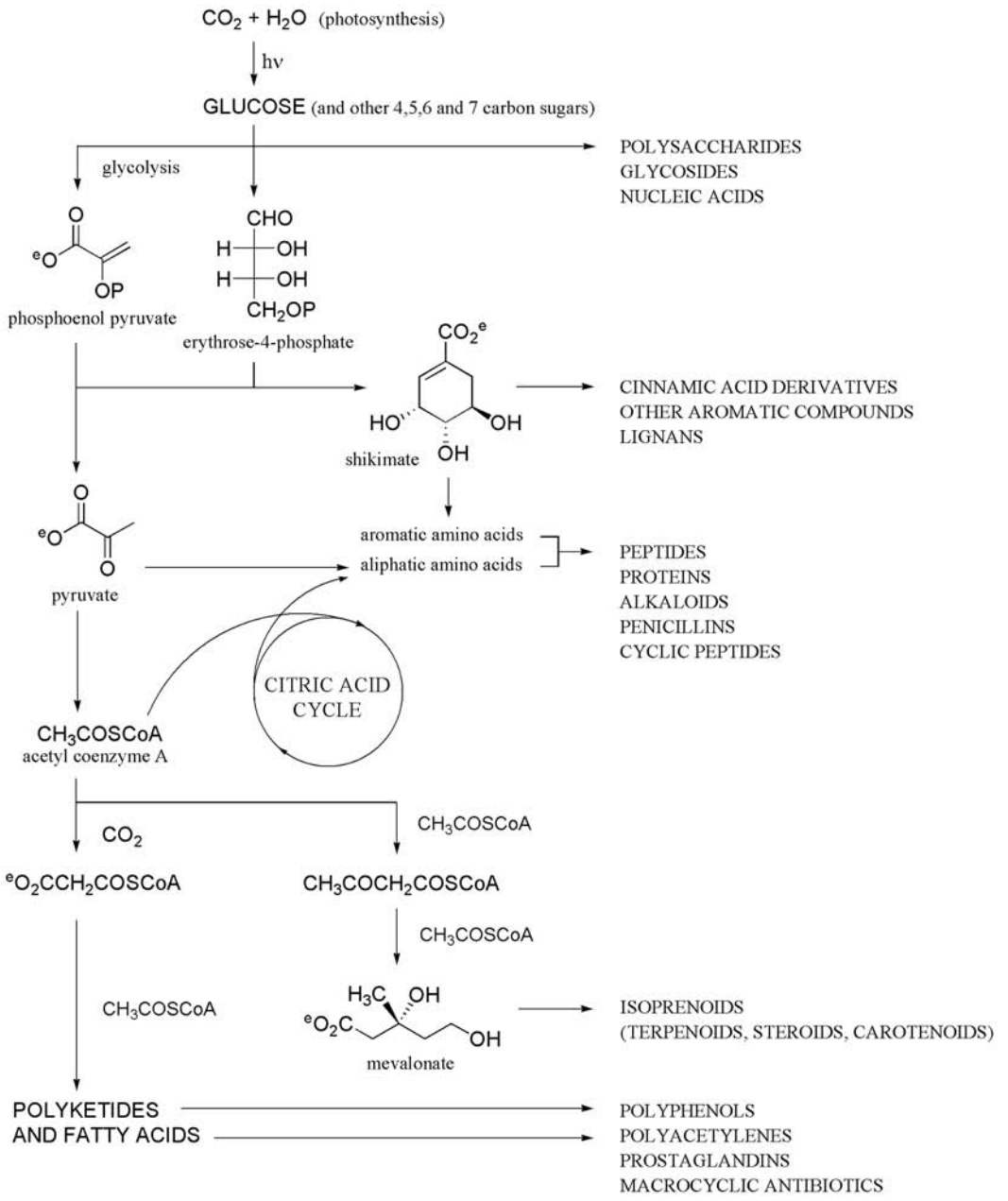


FIGURE I — Biogenesis of secondary metabolites. (Figure from "Chemical Aspects of Biosynthesis," edited by John Mann [1994; fig. 1.2, p. 3]; reproduced by permission of Oxford University Press).

The metabolites of interest here can be classified according to the biosynthetic pathways that give rise to them. These are: 1) the polyketide, 2) the isoprenoid, and 3) the amino acid pathways. The polyketide pathway starts with acetyl-CoA and gives rise to polyketides, polyphenols, and fatty acids. The acetyl-CoA provides two-carbon units that can be linked together head to tail to form polyketides. The polyketide pathway can give rise to linear chains, which then can give rise

to polyphenols through cross-coupling. There are also shikimic acid derivatives that have numerous hydroxyl groups. Fatty acids have acetyl-CoA as a starting point and through a complex series of reactions a linear chain is formed from a number of two-carbon subunits. The isoprenoid pathway again starts with acetyl-CoA, three units of which condense to form mevalonic acid, which gives rise to 5-carbon isoprene (2-methyl-1,3-butadiene) units. The isoprene units are linked up to form terpenes, steroids, carotenoids and similar compounds. The terpenes are classified according to the number of subunits in a way that sometimes confuses the beginner. A monoterpene consists of two isoprene units each with five carbon atoms. Therefore, the series goes: hemiterpenes (5 carbon atoms), monoterpenes (10), sesquiterpenes (15), diterpenes (20), sesterterpenes (25), triterpenes (30), etc. The amino acid pathway derives from various precursors in the Krebs cycle, the glycolytic and shikimic pathways, with some intermediate steps. These are nitrogenous compounds, and in general the nitrogenous secondary metabolites called alkaloids are derived from them. Amino acids also form peptides, which are sometimes cyclic ones, and a fair number of these are of interest from the point of view of chemical defense. The sacoglossan *Elysia rufescens* contains a peptide, Kahalalide F (**Atlas 652**), which has anti-tumoral properties and has been undergoing clinical tests (Hamann, Scheuer & Paul, 1994).

Returning to Figure I, we see that the glycolysis pathway gives rise to acetyl-CoA, which, as we just said, forms polyketides and the mevalonic acid that begins the isoprenoid pathway. Primary metabolites within the glycolysis pathway give rise to several amino acids. 3-phosphoglyceric acid gives rise to the amino acid serine, which in turn is modified into two other amino acids, glycine and cysteine. Pyruvic acid, which is transformed into acetyl-CoA at the end of the glycolysis cycle, is also modified into three amino acids: valine, alanine, and leucine. Acetyl-CoA also enters into the Krebs cycle. Within the Krebs cycle there are two primary metabolites that are modified to form amino acids. Oxaloacetic acid is first transformed into aspartic acid, which in turn may be modified to form isoleucine, methionine and lysine. 2-oxoglutaric acid gives rise to three amino acids through a linear series of reactions: glutamic acid, then ornithine, then arginine. The other three primary amino acids (phenylalanine, tyrosine and tryptophan) are all cyclic. They are produced from shikimic acid, which is formed by the unification of phosphoenolpyruvic acid from the glycolysis pathway with erythrose-4-phosphate from the pentose phosphate cycle. The shikimic acid pathway does not occur in animals, so we have to derive these amino acids from our food.

Making use of such simple starting materials, organisms are able to build up a vast variety of secondary compounds by combining subunits and variously modifying them by altering their composition and rearranging the parts. To understand how this is accomplished it will help to consider a series of examples. The particular examples have been chosen partly because they are relevant to what is said in later chapters.

Let us begin with a fatty acid, stearic acid, $CH_3(CH_2)_{16}COOH$. It is a saturated fatty acid (the sort that one is admonished to eat less of) meaning that it has no double or triple bonds between the carbon atoms. Like other acetogenins it is synthesized from acetyl-CoA units. Two carbon atoms are added at each step, the details of which are too complex to concern us here. The reactions are facilitated by systems of enzymes called fatty acid synthases. The elongate stearic acid molecule that results can then be modified in various ways. For one thing, further two-carbon units can be added. Or the fatty acid can be desaturated, so that there are double or triple bonds between carbon atoms at various positions in the chain. Arachidic acid, $CH_3(CH_2)_{18}COOH$ has the same number of carbon atoms as does arachidonic acid, with four double bonds, and eicosapentenoic acid with five double bonds. Eicosanoids and prostaglandins, which are formed from arachidonic and eicosapentenoic acid derivatives, are of considerable interest for the natural products chemistry of marine animals (see De Petrocellis & Di Marzo, 1994). Although originally found in human

prostate secretion, eicosanoids are widely distributed in animal tissues. They often have a signaling function. Some opisthobranchs synthesize them and use them defensively. Others defend themselves with eicosanoids derived from food. Acetylenic fatty acids, i. e., ones with triple bonds between at least two of their carbon atoms, are another important class of biologically active molecules. Not uncommon defensive metabolites in marine sponges and algae, they occasionally occur in the opisthobranchs that feed upon them.

Head-to-tail condensation of acetate subunits can also produce polyketides characterized by their oxo-groups. These readily undergo cyclization, as we shall see, but in some cases the oxo-groups are readily made out in larger molecules. The biosynthesis of polyketides, which is effected by systems of enzymes called polyketide synthases, is very similar to that of fatty acids (Staunton & Weissman, 2001). Polyketides that are not biosynthesized from acetyl-CoA are most unusual, but for that very reason they need to be discussed here. Certain bacteria, opisthobranchs and fungi have been thought to synthesize polypropionates, which are polyketides that consist of propionic acid units made from propionyl-CoA. In the fungi, it turns out that the molecules in question are not made that way after all (Pedras, Soledade & Chumala, 2005). They are polyketides formed from acetate and then methylated, with the methyl groups derived from the amino acid methionine. The fungi did not incorporate propionate into the metabolites in question. The original evidence for the opisthobranch molecules being polypropionates had been some experiments in which it was shown that propionate labeled with a reactive isotope was indeed incorporated into the molecules (Ireland & Scheuer, 1979; Di Marzo, Vardaro, De Petrocellis, Villani, Minei & Cimino, 1991; Vardaro, Di Marzo, Marin & Cimino, 1992; Cimino, Fontana, Cutignano & Gavagnin, 2004). The possibility had not been excluded, however, that the propionate was degraded into acetate, then condensed into a polyacetate, and finally methylated, as in the fungi. Therefore some experiments using propionate labeled appropriately with ^{13}C were carried out, providing compelling evidence that the propionate units are incorporated intact (Cutignano, Fontana & Cimino, 2009). Propionate biosynthesis in opisthobranchs is therefore like that of bacteria. In a few cases to be mentioned later, there is evidence for opisthobranchs using butyrl-CoA in the biosynthesis of secondary metabolites.

Alkaloids may be roughly characterized as nitrogenous compounds derived from amino acids (Christophersen, 1985a). For example, tryptamine (from tryptophan) combines with pyruvic acid to form 1-methyl-β-carboline. However, alkaloids are often synthesized from other precursors such as isoprenoid compounds (Roddick, 1980) and purines (Misra, Luthra, Sing & Kumar, 1999). Leete (1983) discusses the problem of how the term "alkaloid" should be defined, given that any definition tends to exclude some of them. Pelletier's definition "a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms" is the one that Leete prefers, but he notes that it excludes, for example, such acyclic compounds as spermine. In terms of formal logic, it would seem that the term "alkaloid" is a cluster concept or a term that is disjunctively defined. The alkaloids that do not have the defining properties shared by all of the "typical" ones are included in the class by allowing for exceptions and alternatives. Alkaloids are very common in terrestrial plants, and include such familiar products as strychnine, caffeine, and nicotine. In marine environments they are largely produced by bacteria, often ones that live within the bodies of various animals, such as sponges. The alkaloids of marine organisms often contain halogen atoms, usually chlorine or bromine. The famous "purple of Tyre," a dye that has been extracted from marine mollusks since antiquity, is a dibromo derivative of indigo (Christophersen, 1983).

As is common knowledge, proteins are polymers made up of amino acid subunits. Although proteins are synthesized by the ribosomes, using DNA as a template, many of the shorter amino

acid polymers are made without the participation of ribosomes. Rather there is a protein template. The peptides thus produced sometimes function as antibiotics (bacitracin is a familiar example), as do peptides made using ribosomes and DNA. Peptides having biological activity are of some importance in marine environments. Janolusimide (**Atlas 600**), from the nudibranch *Janolus* (**Photo 119**), is one example (Sodano & Spinella, 1986). Like all tripeptides it was obtained by coupling three amino acids, in this case N-methylalanine, 4-amino-3-hydroxy-2-methylpentanoic acid, and a cyclic hydroxyamide.

Part 2. Biotransformation

Natural products chemists often describe the "total synthesis" of a secondary metabolite. In principle that means that they have made it in the laboratory, starting with the elements of which the molecule is composed. In practice they do not bother to carry out all those steps themselves, but begin at a somewhat later stage, as has been explained in an amusing paper by Cornforth (1983). Likewise, "*de novo*" biosynthesis of a secondary metabolite does not mean that the organism begins with elemental carbon, hydrogen, oxygen and the like, but with primary metabolites. What is called "biosynthesis" may involve taking molecules apart as well as putting them together, and changing their structure as well as adding or replacing components. "Biotransformation" means altering the molecule. It is primarily of interest when the properties of that molecule are changed in a way that affects the biology of the organism. There are various reasons why a metabolite might be transformed in this manner. For example, a more stable metabolite might be stored more easily than a less stable one. This is probably a minor advantage, at least relative to two other ones: detoxification and enhancement of the toxic effect.

Detoxification. An animal that feeds upon another animal or a plant that contains a defensive metabolite obviously needs to avoid any damage that the metabolite might inflict upon it. There are various ways of accomplishing that. One of the most straightforward is selective feeding, mainly by avoiding the part of the food item in which the defensive metabolite is most concentrated. However, there are no documented examples of that for opisthobranchs. Once the food has been ingested, it may be possible to avoid absorbing the defensive metabolite from the digestive tract. If it does make its way into the body, it can be excreted via the kidney. Or the physiology of the animal may be such that the metabolite does little harm.

The nudibranch *Hypselodoris orsini*, a pair of which are shown in **Photo 95** (also called by its junior synonym *H. coelestis*, and often misidentified as *Glossodoris tricolor*), provides an example of such detoxification (Cimino, Fontana, Giménez, Marin, Mollo, Trivellone & Zubia, 1993). This Mediterranean sea slug occurs in association with a sponge referred to in the literature as *Cacospongia mollior* (actually *C. scalaris* and more recently called *Scalarospongia scalaris*) (Demospongiae: Dictyoceratida: Thorectidae), upon which it feeds. The sponge contains the sesterterpenoid scalaradial (**Atlas 486**), which has a pair of aldehyde groups that are responsible for its activity. It is modified by the slug in two steps. First, it is converted by selective reduction of the aldehyde at C-17 to deoxoscalarin (**Atlas 483**). Then the molecule is converted by oxidation to 6-keto-deoxoscalarin (**Atlas 489**), which is then concentrated in specialized organs (mantle dermal formations) at the surface of the animal's body. The location of 6-keto-deoxoscalarin suggests it is deployed against predators. However, the structure of the molecule suggests that it is biologically inactive, and therefore it has been proposed that the mantle dermal formations are a kind of excretory organ (Avila & Durfort, 1996). This interpretation is questionable. The glands are well positioned for a defensive function, and getting rid of metabolites via the gut or kidney would be much easier. One might reply, perhaps, that they represent an evolutionarily modified form of glands that previously did deploy some kind of defensive metabolite.

Enhancement of toxic effects. Provided that an animal that derives defensive metabolites from food can tolerate them, the metabolites can be used in that animal's own defense, and there are various ways in which the defense can be rendered more effective. One way, already just alluded to, is positioning the metabolites in a part of the body where they will be delivered to the attacking predator with maximal effect and minimal damage. This often means moving the metabolites from the digestive tract to the skin. A second way is increasing the concentration of the metabolite. Third, the molecule can be modified so that it is more toxic (or more distasteful) to predators. A good example of this is the Mediterranean *Oxynoe olivacea*, and some other animals of the order Sacoglossa (**Photo 27**). They feed upon a green alga, *Caulerpa prolifera*, in which there is a toxic sesquiterpene, caulerpenyne, with a terminal 1,4-diacetoxybutadiene moiety (**Atlas 206**). The mollusks modify caulerpenyne to the more toxic oxytoxin 1 by selective hydrolysis of one of the two enol acetate moieties (**Atlas 207**). Then they modify it again to form the still more toxic oxytoxin 2 (**Atlas 208**). In general it is not easy to provide compelling evidence for biotransformation. In spite of some experimental evidence that records the absence of caulerpenyne in *Oxynoe olivacea*, in which oxytoxin 1 is compartmentalized in the detachable tail, this biotransformation could be refuted. Some authors (Guerriero, Marchetti, D'Ambrosio, Sinesi, Dini & Pietra, 1993) have suggested that minor dietary metabolites are accumulated selectively, whereas others (Jung & Ponhert, 2001) have hypothesized the biotransformation of the major metabolite mediated by algal lipases during grazing by the slug on the algae. Recently it has been rigorously proved that the biotransformation can be attributed to hydrolytic lipases produced by the slugs, whereas the activity is not present in the alga (Cutignano, Notti, d'Ippolito, Domenèch Coll, Cimino & Fontana, 2004).

Part 3. Deployment of metabolites

In order to explain how opisthobranchs obtain, modify, and utilize secondary metabolites we herein provide some additional details about their basic anatomy. The ancestral gastropod is generally thought to have had a coiled shell and an asymmetrical, twisted body. The gill was located near the front of the animal, over the head, as was the anus. Accompanying the gradual reduction of the shell, the gill and anus have moved backward toward the rear of the body, straightening the animal out somewhat. When a snail gets transformed into a slug, the soft part of the body gets expanded relative to the shell, and the animal loses the ability to withdraw into the shell. At later stages the shell becomes lost or virtually lost, except that in larvae it generally is relatively large and still protects the animal. The muscular foot continues to serve as the main organ of locomotion and extensions of it called parapodia may expand and cover much of the body. The foot allows the animal to move, albeit slowly, from place to place. It also permits the animal to cling to the substrate while moving about or when apt to be dislodged by waves or currents. Such an arrangement is effective for an animal that grazes upon food items that live attached to the surface (sessile organisms) such as plants and sponges, or pursues sedentary or slow-moving prey such as clams and snails.

Combined with this mode of locomotion is a feeding mechanism that allows snails and slugs to subdue and utilize food sources that have effective mechanical defense. Gastropods are generally equipped with a rasp-like structure called a "radula," which may be compared to a tongue covered with teeth. They can grab or scrape with it, usually with the two actions working in tandem. Food is taken into the gut and passed to a stomach, where it usually gets broken down somewhat. Then particles of food are passed to a structure called a digestive gland where digestion occurs intracellularly—i. e., inside the cells that line the gland. Nineteenth-century zoologists called the digestive gland a "liver" but, because it is only superficially similar to that of vertebrates (i.e., the

namesakes are analogous, not homologous), the term is no longer used. However, like the vertebrate liver, it is an important site of detoxification.

The stomach and digestive gland of opisthobranchs have been convenient sources of interesting metabolites for investigation. However, they do not provide very effective defense in that position, and getting them to more appropriate locations is an important evolutionary theme. The metabolites generally become concentrated in the skin of the animal, and in particular locations where they will be most effective. Specialized "repugnatorial glands" are common, and the mantle dermal formations already mentioned are a good example.

Sea slugs are rich in mucus, and may release copious quantities of it when they are attacked. This mucus may contain a substantial amount of secondary metabolites, even when the basic effect on the predator is a mechanical one. Mucus evidently represents the first line of chemical defense, and the repugnatorial glands come into play when mucus is not sufficient to repel the predator and the mollusk is bitten (Mollo, Gavagnin, Carbone, Guo & Cimino, 2005). Another place where secondary metabolites may be concentrated is the gonad, which in opisthobranchs is called the "hermaphroditic gland" because every animal is both male and female at the same time and produces both sperm and eggs. The eggs are enveloped in protective mucus, and it is they (or the layer of nutritive albumen surrounding them), that contain the metabolites.

Chapter II

Comparisons and Experiments

Part 1. Introduction

Epistemology is the branch of philosophy that is often defined as the theory of knowledge. It considers the question "How do we know what is true?" Among scientists, practical epistemological issues are often discussed under such rubrics as methodology and experimental design. When scientists start writing polemics about methodology it usually is not a good sign, because it suggests that they cannot find a straightforward means of settling their differences. In this chapter we try to present a straightforward, yet philosophical, explanation of how research in the areas of interest is carried out and why it is done that way. Scientific research is not easy, and we illustrate some of the difficulties and pitfalls with examples drawn from our own research as well as that of others. On a more positive note, there are also examples of ingenious experiments and clever arguments from which valuable lessons can be learned.

Part 2. Systematics

Opisthobranchs of the order Anaspidea are called "sea-hares" because of their fanciful resemblance to a bunny (**Photo 17**). The comparison has never fooled anybody. But it illustrates the point that much more is involved in classification than just putting similar objects together. Scientists go out of their way to avoid being deceived by appearances, and this is especially true of systematic biologists, who often get burned by them. They need to sort their materials out into groups of objects that correspond to objective reality, and that is not always easy. The problems are notoriously difficult even at the most basic level of classification: the species. Here we will evade the controversial aspects of what it means to be a species by referring the interested reader to the inevitably long and difficult book that one of us has written on such topics (Ghiselin, 1997).

In evolutionary biology, a species is a reproductive community that is held together by sex.

Such units are important because they evolve. That means that they can become transformed through time. It also means that they can undergo only a limited amount of diversification because sexual reproduction tends to homogenize the group. Within species, diversity often takes the form of local populations that are supposedly adapted to local conditions of existence. But if evolutionary diversification is to go further, it is necessary for the populations to become reproductively isolated from one another—in other words to become separate reproductive communities even when they are located in the same area. When that happens speciation has occurred, and the populations can continue to diversify, forming genera, and, through repeated speciation events, families, orders, and higher groups. Each node in a phylogenetic tree corresponds to a speciation event.

Organisms belonging to closely related species tend to differ somewhat in their important properties, including such ecological ones as diet and microhabitat. Sometimes they have "diagnostic" characteristics that make it fairly easy to identify a specimen as belonging to one species or another. Life, however, is not always that simple, and what really matters is not whether we can identify organisms to species but whether the organisms themselves can do so. Organisms that look alike to us may have important differences. Since these differences are often matters of physiology or behavior, perhaps with respect to what they feed upon and what metabolites they may utilize, they may be of particular significance to the natural products chemist. Species that are hard for us to tell apart are called "cryptic species" and generally these are closely related. They are particularly common in several groups of marine animals, and in some of these the number of species turns out to have been underestimated by a factor of ten (Knowlton, 1993). Such an underestimate can give a serious misconception of ecological importance and evolutionary rates within a group. It also distorts our understanding of how new species are formed and of the role of speciation in evolution. The detection of cryptic species has been facilitated by modern genetical techniques, which provide a better index of physiological differentiation. It also may be possible to detect them by means of behavioral studies and genital anatomy.

The other side of the coin is species within which there is a great deal of variation, whether local or otherwise. Opisthobranchs often have what are described as "color morphs" within a species (Ros, 1976). They may have very different color patterns. We have already mentioned another evolutionary phenomenon that may prove misleading. This is "mimicry," in which animals of one species come to resemble those of another because the resemblance as such affords protection or some other advantage. Such mimicry is not uncommon where chemical defense is involved. As was mentioned above, in Batesian mimicry an unprotected "mimic" resembles a protected "model," whereas in Müllerian mimicry two or more protected forms advertise their distastefulness using the same pattern. Both kinds of mimicry have been found in opisthobranchs (Gosliner & Behrens, 1990; Gosliner, 2001). **Photo 59** shows the close resemblance between the dorid nudibranchs *Chromodoris geometrica* (above) and *Phyllidia pustulosa*, which are in different families. In the case of certain insects, members of the same species mimic different models, and this polymorphism may occur locally as well as geographically.

Misidentifying an organism of interest can have serious consequences for scientific research, and our own experience provides a good example. The animal that had been known to local naturalists as *Cylindrobulla fragilis*, shown in **Photo 25**, was found to have some very interesting natural products (Gavagnin, Marin, Castelluccio, Villani & Cimino, 1994). Because *Cylindrobulla*, shown in **Photo 24**, is considered to be very close to the common ancestor of the order Sacoglossa, this creature was of particular interest in our effort to reconstruct the evolutionary history of that group. However, we realized that the identification had been made from the shell. Upon checking out the internal anatomy, it became obvious that we were dealing not with *Cylindrobulla*, but with *Ascobulla*, which is not even in the same family, although perhaps it should be. The shell has

evolved much more slowly than the feeding apparatus. Fortunately we found this out before we published our review article (Cimino & Ghiselin, 1998).

Our interpretation of evolutionary history depends on our knowledge of the relationships between the various lineages and groups of lineages sharing common ancestors (clades) that make up the genealogical, or phylogenetic, tree. Techniques for working out the relationships have been the subject of a vast technical literature. Our aim here is to provide an introductory treatment that will allow our readers to understand what is said later on. Of particular importance is the point that various classifications are available in the literature, and that although these tend to reflect the genealogical relationships, the correspondence may be only approximate, especially in what we may call "traditional" systems. With the passage of time, classifications are being revised so as to make them come closer to the genealogical ideal, but this is work in progress.

Merely putting those animals together that have characters in common often gives misleading results. As we have mentioned, the opisthobranchs have repeatedly evolved from snails into slugs. If we divided them into two groups, snails and slugs, the latter assemblage would not share a common ancestor, except in the trivial sense that all the organisms in the world share a common ancestor if one goes far enough back in time. A group (*taxon*) consisting of all slug-like opisthobranchs would be called a "polyphyletic" one, meaning that it is made up of several lineages. The other assemblage, consisting of opisthobranchs minus those that have become slugs, would be called a "paraphyletic" taxon. They are, respectively, *polyphyletic* and *paraphyletic grades*, rather than *clades*. Clades are complete branches of the tree, including a common ancestor and all of its descendants. A taxon that corresponds to such an entire branch is said to be (strictly) *monophyletic* or, by some authors, *holophyletic*.

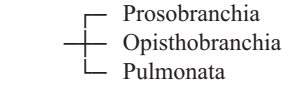
The gastropods (including both snails and slugs) as a whole are thought to constitute a single branch of the animal tree and form, therefore, a monophyletic taxon, the class Gastropoda. All of them share a unique, derived character (synapomorphy) that indicates a single origin for the group. This is "torsion" (see Ghiselin, 1996a). Not to be confused with the snails' coiling, it is a rotational movement of the upper part of the body, including the gills and the anus, from the posterior part of the body to the anterior, with the result that the body of the animal is asymmetrical. Torsion can be observed in developing embryos.

In opisthobranchs there is partial detorsion, so that the gills and the anus get displaced backwards again and bilateral symmetry is partly re-established, although as we have just noted, traces of torsion are still detectable in the embryos and larvae, which still have a shell, and also in some internal organs. The result of detorsion is obvious in dorid nudibranchs such as *Chromodoris quadricolor*, shown in **Photo 73**. Here, the anus and gills have returned to the posterior end of the body, though the openings of the reproductive system are near the anterior end, on the right side. It is conceivable that torsion may have occurred more than once (things like it occur in other groups). The reason why torsion is thought to have occurred only once in the evolution of gastropods is that it does not conflict with other characteristics. When there are such conflicts it is assumed that the correct relationships are those that represent the least number of evolutionary innovations. This is called the "principle of parsimony" and we will say more about it later.

Traditionally, and the tradition goes back to the early nineteenth century, gastropods have been divided into three major groups: the sub-classes Prosobranchia, Opisthobranchia, and Pulmonata. The original idea, which seems rather quaint from our modern point of view, was to have important subdivisions that would be based on properties of the respiratory apparatus. The prosobranchs would have the original condition, with the gills pointing forward (Gk. *proso*- front). The opisthobranchs would have various degrees of detorsion, such that the gills would be near the rear of the animal (Gk. *opistho*- rear). And finally, the pulmonates would have a lung instead of gills (Gk.

pulmo- lung). (Actually one of the originally paired gills is generally lost, so that an opisthobranch has only one of them, and some pulmonates have a gill in addition to a lung.)

This higher-level classification has persisted to the present day. It does sort the gastropods out into three more or less distinct groups. However, it illustrates some of the problems with traditional classifications. If we tried to interpret it as a tree, we would get a "trichotomy" as follows:



The diagram suggests that the common ancestor of Gastropoda split up into three species, each of which gave rise to one of the three subclasses. From what we can tell, things were very different. Pulmonata does seem to be a single lineage, in other words a monophyletic taxon. At least we can make a case for that on the assumption that the mantle cavity, a depression that surrounds the gill in many gastropods, was converted into a lung just once, or at least that the transition to breathing air happened in that lineage alone. The presence of a lung is a derived character (synapomorphy) that is shared by all pulmonates and is not inherited from their forebears. It is somewhat more difficult to find a good synapomorphy for Opisthobranchia, though the folded gill that characterizes many of them might be invoked. Part of the trouble here is that Pulmonata and Opisthobranchia are very closely related. For that reason they are often placed together in a larger assemblage called "Euthyneura," so called because the twisting of the nervous system that took place as a result of torsion is no longer so obvious. Quite a number of rather obscure characters have been used as synapomorphies for Euthyneura. Unfortunately they often occur in some other gastropods. They include a peculiar larval shell, a spermatozoon with a keel, and the hermaphroditic reproductive system and some of its features. But Prosobranchia shares only the ancestral characters inherited from the gastropod common ancestor, and Euthyneura is closer to some prosobranchs than to others. In other words, Prosobranchia is a paraphyletic grade. In such modern classifications as that of Ponder and Lindberg (1997) Euthyneura, together with its closest relatives forms a monophyletic group called Heterogastropoda. Within the Euthyneura the trees give somewhat ambiguous results and the classification remains in a state of flux.

Might Opisthobranchia or Pulmonata turn out to be paraphyletic? This seems a distinct possibility in view of the modest amount of change that would have to be involved in transforming something like a primitive opisthobranch into a pulmonate. Pulmonates might easily be more closely related to some opisthobranchs than others. Indeed there was a brief period when it was claimed that some marine pulmonates are really not pulmonates at all, but opisthobranchs. It turned out, however, that the opisthobranch characters on which this claim was based were merely symplesiomorphies, showing only that pulmonates are gastropods (Ghiselin, 1996b). Unfortunately the confusion created problems for the natural products literature when metabolites from pulmonates were said to be from opisthobranchs (Ireland & Faulkner, 1978).

The basic procedure, then, in reconstructing genealogical relationships, is to put the animals together on the basis of shared, derived characters. But we run into difficulties in such apparently unproblematic issues as what is a character, and how we know that it is derived. It is more or less obvious that the habit of breathing air and the presence of a lung in pulmonates represent derived conditions. We can tell this partly by outgroup comparison: mollusks in general are aquatic animals with gills. But we also know that life originated in the sea, and that very few lineages have returned to it. One reason why somebody might come up with a classification that puts slugs together into a polyphyletic assemblage is that so many parts of the body are involved. It is not just the shell, but other parts that are associated with it, that get lost. For example, in the generality of prosobranchs

the opening of the shell is closed by a door-like structure, the operculum. According to the principle of parsimony, the best tree is the one that invokes the least number of character changes. In such reasoning we would want to avoid counting the same character twice, and having physiologically-correlated features or something linked to the same environment might give us a misleading impression. Therefore it is reasonable to include evidence from a wide range of body parts and organ systems.

Extending that same line of reasoning we might resort to properties that are supposedly far removed from the anatomy and the environmental influences that might give us a misleading impression. During the last few decades, molecular data have been increasingly used, not necessarily as a replacement for, but quite often as a supplement to, the traditional evidence. We might mention the use of chromosome numbers and chromosome morphology as an early precursor of the molecular approach. Within the opisthobranchs there is an apparent clade, consisting of Nudibranchia plus Notaspidea, that can be characterized by a distinct drop in chromosome numbers from an initial 17 (haploid) to 12 or 13 (Inaba, 1961; Patterson, 1969; Vitturi, Rasotto, Parrinello & Catalano, 1982; Curini-Galletti, 1985, 1988; Wägele & Stanjek, 1996). Most of the new evidence, however, comes from sequence data, either of nucleic acid polymers (RNA and DNA) or proteins.

Since the work of Field, Olson, Lane, Giovannoni, Ghiselin, Raff, Pace and Raff (1988) on the phylogeny of the animal kingdom, the preferred molecule has been 18S (small-subunit) rRNA. Ribosomes play a crucial role in protein synthesis, and are highly conserved. They evolve slowly, but enough changes appear that they have proven very useful for studying relationships that are not very recent. Mitochondria contain somewhat smaller ribosomes, and their 16S mRNA, which evolves somewhat faster, is more useful for more recent relationships. The ribosomal RNA or the corresponding DNA is sequenced using electrophoretic techniques, revealing the order of the nucleotides of which the molecule is composed. The next step is to "align" the molecules from the different organisms of interest, so that what appears to be the same site has identical nucleotides if no evolutionary change has taken place, or different ones, if one nucleotide has been replaced by another. Some aligned sequence data from Rebecca Johnson's work-in-progress on dorid nudibranchs of the family Chromodorididae are shown in Table 1. The nucleotides at several of the sites are all identical, indicating no evolutionary change. In *Hypselodoris kanga* there are two alignment gaps, where no nucleotide is present. This probably means that a pair of them has been lost. Nucleotides may also be gained. It is possible to misalign the sequence, and that may create errors, both major and minor.

TABLE 1. Some aligned sequence data from Rebecca Johnson's work-in-progress on dorid nudibranchs of the family Chromodorididae.

Risbecia tryoni	CCTAAATAATTATTTGAAGTT
Risbecia tryoni	CCTAAATAATTATTTGAAGTT
Hypselodoris bertschi	CTTAACTAATTATTTGAAATT
Hypselodoris maritima	CCTAACTAATCACTTGAAATT
Hypselodoris cf. *nigrolineata*	CCTTATTAATTAATTGAAATT
Hypselodoris kanga	CCTTA-TAATTATTTGAA-TT
Hypselodoris infucata	CCTTAATAATTATTTGAAATT
Hypselodoris obscura	CCTTAATAATTATTTGAAATT
Hypselodoris obscura	CCTTAATAATTATTTGAAATT
Hypselodoris kaname	CCTTATTAATCATTCGAAATT
Hypselodoris paulinae	CCTTATTAGTCATTCGAAATT
Hypselodoris bollandi	CCTCAACAATTATTCGAAGTT
Hypselodoris jacksoni	CCTGATTAATTATTTGAAATT
Hypselodoris jacksoni	CCTGATTAATTATTTGAAATT
Hypselodoris regina	CCTGATTAATTATTTGAAGTT
Hypselodoris reidi	CCTGATTAATTATTTGAAATT
Hypselodoris zephyra	CTTAATTAATTATTCGAAGTT
Hypselodoris zephyra	CTTAATTAATTATTCGAAATT
Hypselodoris zephyra	CTTAATTAATTATTCGAAATT
Noumea crocea	CTAAACTATTTAGTTGAATTT
Noumea flava	CTAAATTAATTATTTGAAGTT

Once the nucleotide data have been aligned, the next step is to do some kind of statistical analysis that will estimate the best tree. There are

various ways of doing that, but the basic one is parsimony analysis, which minimizes the number of evolutionary steps needed to produce a tree on the assumption that the tree with the smallest number of changes is the one least likely to be false. Because, however, the approach is basically a statistical one, the picture that we get is inevitably somewhat distorted by chance as well as other things. The more likely events obviously occur more often, but the less likely ones occasionally happen too. On top of that the approach works better if the molecules have been evolving at the same rate, which is by no means always the case.

The study of Field, Olsen, Lane, Giovannoni, Ghiselin, Raff, Pace and Raff (1988) provides some examples of what happens to the available body of evidence when molecular results are added. In general it tended to confirm traditional views, but there were some surprises. First, the animal kingdom seemed not to be monophyletic. The first branch, or coelenterates, seemed to be closer to plants than to other animals. As it turned out, the result was not statistically significant. Without enough characters, the coelenterates had slipped off onto an adjacent branch, but then again they had not gone very far. Something similar happened to the mollusks, but for a slightly different reason. The group broke up and its subunits became associated with several related lineages. Such a phenomenon had already been anticipated, and is very easily explained. The mollusks and their relatives diversified very rapidly and there was insufficient time for enough changes in the molecule to reveal what had happened. Three other interesting, and to some degree unexpected, results are worth noting. First, the brachiopods, which had been widely considered relatives of chordates and echinoderms were far removed from that position, and much more closely related to mollusks and annelids. This finding has been consistently supported by later evidence. Second, the arthropods turned out to be less closely related to annelids than conventional reasoning would have had it. Again this has been confirmed by subsequent research, which led to the discovery of a hitherto unexpected group, Ecdysozoa, consisting of arthropods, nematodes, and some others (Aguinaldo, Turbeville, Linford, Rivera, Garey, Raff & Lake, 1997). And finally, the mollusks, annelids, brachiopods and some other groups turn out to form a unit called Lophotrochozoa. Supporters of the flatworm theory of molluscan origins lost this round in the long debate, and opinion has swung in favor of the annelid theory (Ghiselin, 1988; Lindberg & Ghiselin, 2003).

The lesson to be learned from such experience is that although ribosomal RNA or protein sequence data can be compelling when the issue is the approximate position of a group within the tree, the exact branching sequences are generally too close to call. Supposedly, one can hope to get more compelling results by sampling a larger part of the ribosome (28S rRNA), by adding protein sequence data, and of course by using the traditional "morphological" or better, anatomical, characters. There is another possibility, however, and that is mapping, and comparing, entire genomes, or at least large parts of them. The basic technique was first applied to the comparison of chromosome maps of *Drosophila* (Dobzhansky & Sturtevant, 1938). Recent studies are sometimes based on the sequence of genes in mitochondria, which have a small genome of their own (Grande, Templado, Cervera & Zardoya, 2002; Kurabayashi & Ueshima, 200). Groups of genes occasionally move from one place to another within the genome (translocation). In other cases a portion of the genome is turned around so that it faces in the opposite direction (inversion). The rationale for using inversions as evidence for relationships is that it is highly improbable that an identical inversion will evolve more than once. A part of the chromosome has to be detached at two identical sites, and then reattached. This approach has given some very valuable results, and we will discuss some of them below. It has the obvious drawback of not working within lineages in which there have been no rearrangements.

Part 3. Metabolite Chemistry and Physiology

Modern techniques make it possible to detect and identify compounds in very small quantities. Mass Spectrometry (MS) and nuclear magnetic resonance (NMR) are particularly appropriate for characterizing the structure of organic compounds such as secondary metabolites. MS gives complete information on the elemental composition of the molecule. NMR makes it possible to connect all of the atoms, in particular the hydrogen and carbon atoms, in the structure of interest. The NMR and sometimes the MS techniques require getting the materials into solution and this task is not as easy as it might sound. The solvent has to be selected so as to avoid interactions with the extractable compounds that could lead to artifacts. Naturally it is difficult to prove that a compound from an extract is identical to that present in the natural source. To avoid such complications it is important to minimize the number of steps between the extraction of the material and the isolation of pure compounds. One useful stratagem is to record the NMR spectrum of the entire extract. That would provide a fingerprint of all the compounds that are extractable from the natural source.

When chemists search for secondary metabolites the usual technique has been to extract the whole organism using a solvent such as acetone or methanol and see what turns up in the solution. Any metabolite that looks interesting may then be purified and characterized. Such a whole-body approach may be all that is necessary if the goal is merely to discover previously unknown compounds, but otherwise it is only a beginning. It does not tell us where the metabolite was located in the body. The gut is apt to contain a variety of metabolites derived from food, only some if any of which are used defensively, but their detection at least gives something of an idea of what the animals eat and what their foods contain.

To get a better picture of what is going on within the animal as a whole, it is often appropriate to separate out the various parts of the body in the expectation that metabolites will be concentrated in the places where they are stored and deployed. They may, for example, be more or less restricted to the skin, where they are in a good position to protect the animal from predators, or in the ovarian tissue, indicating that they protect the eggs, developing embryos and larvae. It may be problematic whether the presence or absence of a given metabolite in a sample actually represents the physiological situation in the organism. The molecules may undergo chemical reactions. Some compounds detected by chemists have turned out to be degradation products. Intermediates in a series of synthetic reactions may not be present all the time, or they may exist at very low levels and be hard to detect. A defensive metabolite of interest may be formed only when the animal is under attack. And it may be unstable, as is polygodial (**Atlas 235**).

When metabolites are accumulated and transformed it is generally possible to follow them through the various parts of the body and through the food chain. The transfer of secondary metabolites from prey to predator and to various tissues is merely suggested by the distribution of the metabolites in both organisms. However, the evidence can be quite compelling. For example it was found that the nudibranch *Hypselodoris webbi* (now *H. picta*) eats sponges of the genus *Dysidea* (Demospongiae: Dictyoceratida: Dysideidae). A specimen of *Dysidea fragilis* was collected from Mar Menor in Spain and offered to *Hypselodoris picta* **Photo 97**, which is altogether absent from that area (Fontana, Giménez, Marin, Mollo & Cimino, 1994). The nudibranch immediately started to eat the sponge and, not surprisingly, it was possible to document the transfer of the sponge metabolite, (-)furodysinin (**Atlas 328**), to the nudibranch and to the repugnatorial glands (mantle dermal formations) where they are well-situated to deter predators, being concentrated near the vulnerable organs (gills and rhinophores).

If an animal is dependent upon its food as a source for metabolites this can be documented by means of experiments in which the food supply is manipulated. Or a kind of natural experiment can be done. Because the availability of food varies from place to place, the metabolites will not always

be present locally. Animals that derive the secondary metabolites from food will therefore tend to vary geographically in both the amount and kind of metabolites that they contain. If, on the other hand, the animals that accumulate the metabolites are able to synthesize them, they are not dependent upon the environment, and the level of metabolites will be constant over a wide geographical area. This kind of evidence was first invoked by Faulkner and his collaborators (Faulkner, Molinski, Andersen, Dumdei & de Silva, 1990) in a study on Eastern Pacific dorid nudibranchs. (See also Kubanek, Faulkner & Andersen, 2000). We should note a couple of complications, however. If the animal is so specialized that it eats only one species of food organism, we should not expect it to vary geographically in its metabolites. And it might vary geographically with respect to what metabolites it synthesizes.

De novo synthesis can be documented experimentally, but it is not always easy to do so and not much work along these lines has been done. The method used is to inject the animal with precursors of secondary metabolites containing an isotopic label, such as radioactive carbon. Alternatively, the transformation of the probe molecule can be tested in rough enzymatic (cell-free) preparations obtained from either whole animals or particular parts of them. If the animal can biosynthesize the secondary metabolites, and if all else goes well, the metabolites will contain the label. Working with a radioactive precursor, the detection of radioactivity in the secondary metabolites proves that this metabolite is biosynthesized by the animal, but not that the precursor itself is incorporated, for the possibility that radioactivity has contaminated some catabolites is not excluded.

Although less sensitive than radioactive labels, stable isotopes give less equivocal results because their precise position in the molecule can be determined and the label does not contaminate other molecules (Cimino, Fontana, Cutignano & Gavagnin, 2004). Different atoms in the precursor can be labeled. Doing so provides a way of showing how the parts of the primary metabolite molecule or other precursor get incorporated into the secondary metabolite molecule. That may help to reveal the intermediate steps through which biosynthesis takes place.

It is generally understood that biosynthetic processes are under the control of enzymes. This aspect of secondary metabolite biosynthesis has been extensively studied in terrestrial plants. Unfortunately we have very little information about the role of enzymes in the biotransformation and biosynthesis of secondary metabolites in mollusks. The glycosidases of the anaspidean *Aplysia fasciata* have, however, been characterized (Giordano, Andreotti, Mollo &Trincone, 2004). Lipase activities have also been described for the sacoglossan *Oxynoe olivacea* (Cutignano, Notti, d'Ippolito, Domènech Coll, Cimino & Fontana, 2004). These lipases are very selective. Cyclooxygenase is known to play a role in the synthesis of prostaglandins by *Tethys*.

The secondary metabolites of terrestrial plants are familiar to us largely because of their effects on human beings and other animals. Many are responsible for the chemicals in flowers that attract insect pollinators and are used in perfumes. Plants such as garlic and a host of herbs that we raise in our gardens to flavor our meals owe their distinctive tastes to chemicals that repel insects. Likewise, the narcotics and alkaloids that are used in medicine and that people often abuse are there because they protect the plants that produce them. It stands to reason that pharmacologists and others have devoted a great deal of effort to studying such metabolites, and that much is known about how they affect our bodies and those of insects. Marine natural products, however, do not provide us with important flavorings, perfumes, pesticides, narcotics or stimulants. There are quite a number of marine organisms that kill human beings, but they have attracted attention mainly as things to avoid. Research aimed at finding new drugs from the sea has largely involved screening of metabolites for possible activity as antibiotics, tumor-suppressants and the like. When such activity is found, the compound is studied to see whether it, or something like it, is promising for medicinal use.

There has been very little work, from a chemical point of view, on how the secondary compounds of marine organisms produce their effects on the animals that eat them. An interesting example of what can be done along those lines is a study by Caprioli, Cimino, Colle, Gavagnin, Sodano and Spinella (1987) on what we might call the "business end" of dialdehyde compounds with an adjacent double bond, such as polygodial (**Atlas 235**). These generally have a 1,4-conjugated aldehyde moiety, which is able to react with primary amines. The effect at the molecular level was established by comparing the effects of a series of analogs, some natural, and others synthetic. It depends upon the distance between the two aldehyde groups. This molecular configuration results in a peppery taste, and many plants, such as peppers, as well as fungi have metabolites with it (Jonnassohn, 1996). As to marine organisms, conjugated 1,4-dialdehydes have been detected not only in opisthobranchs but also in pulmonate gastropods, sponges and algae, where they are present in protected form as bisenolacetate. Some nudibranchs are able to biosynthesize their dialdehydes, some to modify those present in sponges so as to reduce their toxicity, and some to hydrolyze the algal enolacetates to improve their protective efficacy.

Part 4. Metabolite Ecology and Adaptive Significance

As we mentioned earlier, the notion that secondary metabolites are excretory products or otherwise non-adaptive components of the organism is no longer taken seriously. This does not mean, however, that their adaptive significance is uncontroversial. On the contrary, there have been lively debates about such matters, and the arguments will no doubt continue. When we say that secondary metabolites are "defensive" it should be taken only as a broad generalization. Many might better be called "offensive" rather than "defensive" and we have to include actions against competitors as well as predators as part of the larger picture. Furthermore, many secondary products play important roles in reproduction, species recognition, and other important biological activities. Even where a given metabolite does repel predators, there is no reason why it cannot do something else as well, such as attract potential mates. And some of the secondary compounds are only indirectly connected with the biologically active ones. They may be precursors, byproducts, protected forms of the molecules, the products of detoxification, or molecules that have become altered while being processed for chemical study. Therefore it is important to have criteria for establishing the adaptive significance of the metabolites.

Simple experiments often provide convincing evidence that a metabolite renders a food item distasteful or toxic. The usual procedure has been to mix some of the metabolite of interest with a solidifying agent such as agar, and then present it to fish, together with a similar preparation that does not contain that metabolite. If the fish rejects the former, but eats the latter, we have positive evidence that when the metabolite is present it can deter at least some predators. Strictly speaking the "food" without the metabolite is not a control, but it does show that its presence or absence makes a difference. Negative results are another matter. Absence of evidence that something happens is not evidence that it does not happen.

A particularly instructive example of negative evidence is provided by the eicosanoids of gorgonian corals. Early workers assumed that the reaction of fishes to defensive compounds would be immediate. However, Gerhart (1984, 1986, 1991), noting that eicosanoids induce vomiting when taken orally by humans, experimented with their effects on fish, and found that they have the same effect. Thus fish might learn to avoid the food item even though it was not distasteful, and even though the negative experience was somewhat delayed. A similar emetic effect has been found in a diterpenoid (**Atlas 355**) that bears the amusing name "pukalide" (Gerhart & Coll, 1993). In unpublished work Arnaldo Marin showed that fishes can memorize the patterns associated with

distasteful chemicals. He offered edible models resembling some opisthobranchs in shape and color to populations of a wrasse, *Thalassoma pavo*. He found that the fish first eats the model without any compound, then refuses otherwise identical models treated with compounds, and subsequently avoids untreated models.

Negative results with experiments on fish might tend to be misleading when the "target" predatora are not fish, but other animals, such as crustaceans or gastropods. It would be like testing the effects of secondary metabolites from land plants on mammals and not bothering to consider their effectiveness as insecticides. There are also a wide variety of fishes, and it might make a difference which of them is chosen as an assay organism. Often it has been just one species, and a freshwater one at that, confined to an aquarium. To make the experiments more realistic, they are often carried out in the field, using a variety of predators considered likely to be important inhabitants of the environment of the organism that is supposedly protected by the metabolite in question. It may also make a considerable difference whether the metabolite is part of a living animal or an artificial bait. Penney (2004) presented specimens of the dorid nudibranch *Cadlina luteomarginata* to various predators. His results showed the predators rejecting the nudibranchs more consistently than in earlier studies using food that had been coated with chemical extracts or single compounds. Evidently the metabolites in the extracts are unstable. Gosliner (2001) offered nudibranchs and sacoglossans to fish of four different species. In many cases the nudibranchs were taken up, but regurgitated, sometimes injured but generally unharmed.

Another pitfall that experimentalists need to avoid is making the assumption that defensive metabolites have to be distasteful to the predators. Some defensive chemicals work by jamming the chemoreceptors of predators (Kitteredge, Takahashi, Linsley & Lasker, 1974).

It is not necessary to "experiment" in the sense of manipulating the organisms to test the hypothesis that a metabolite plays a defensive role. Such an hypothesis gains favor if the metabolite is distributed in such a way that it enhances that effect, rather than being distributed more or less at random in the body. The defensive metabolites tend to be concentrated in those regions where they will first come in contact with the predator. This generally means the surface of the body of the prey. In opisthobranchs they are often concentrated along the rim of the mantle, where an attacking fish is apt to grab them or have a nibble. There is anatomical apparatus that mobilizes the metabolites and deploys them at the predator (repugnatorial glands, or mantle dermal formations) (Garcia-Gómez, Cimino & Medina, 1990; Garcia-Gómez, Medina & Coveñas, 1991; Avila, 2006; Wägele, Ballesteros & Avila, 2006). This effect is reinforced by coloration patterns, often a series of lines at the edge, or spots surrounding the concentrations of glands.

Furthermore, the glands with their advertisements are often positioned in a manner that directs the predator's attack away from places where an attack might do serious harm. **Photo 87** shows a specimen of *Mexichromis macropus* with bright red spots around the slightly uplifted mantle rim. Underneath the red spots, repugnatorial glands (mantle dermal formations) can be seen by transparency. The delicate gill can be seen at the left (posterior) end of the animal. Near the anterior end are located a pair of sensory rhinophores rendered less conspicuous by having the same violet color as spots on the dorsum. The presence of mantle dermal formations is a synapomorphy that differentiates the family Chromodorididae from their apparent sister group, Actinocyclidae and most other dorids (Gosliner & Johnson, 1994). The metabolites are in fact deployed at the time when the slug is hassled by predators: a diver studying opisthobranchs in the field often evokes their discharge when handling the animal. Furthermore, different metabolites are effective against different predators, and against different kinds of attack. Accordingly there may be different metabolites in the eggs (and ovaries) and in the embryos and larvae on the one hand, and in such locations as the skin or mucous secretions of the adult animals on the other.

It would be fallacious to argue against the protective role of a metabolite on the grounds that the delivery system might be improved. The most effective arrangements must have been evolved over a long series of generations, and we would only expect to find intermediate stages in their elaboration. Likewise, one must not be misled by the fact that defensive metabolites are not completely effective against predators. We should expect them to reduce the amount of predation, and to occur together with other means of defense. The opisthobranch *Oxynoe* feeds upon the alga *Caulerpa*, and uses metabolites from it defensively. However, it is well camouflaged on the alga. In **Photo 27**, a specimen of *Oxynoe olivacea* on *Caulerpa prolifera* can be seen exuding a cloud of toxic material. *Oxynoe* can also deal with predators by autotomy. Like lizards that shed their tails when caught, these slugs can break off the end of their foot and crawl away. Such autotomy is quite common in opisthobranchs, and is often accompanied by behavioral and physiological adaptations that enhance the effect. We discuss a few examples in later sections of this monograph.

Many opisthobranchs have escape reactions that are elicited by the attack of a predator. These often involve detachment from the substrate and writhing from side to side allowing the slug to be carried away from the predators by currents. In some cases opisthobranchs have well-coordinated swimming movements due to undulations of the body or the flapping of parapodia. The two orders of pteropods, Thecosomata and Gymnosomata are characterized by the presence of "wings" that allow them to swim very effectively and spend their lives in the open water. This kind of locomotion could easily have been derived from swimming used to escape predators.

Considering the various forms of defense that occur in opisthobranchs from a bioeconomic point of view, there is a complex pattern of costs and benefits that deserves further study. We have noted that there may be several lines of defense, and some of these are more costly than others. Avoiding the attacks of visual predators by some kind of crypsis, such as camouflage or nocturnal habits, is very common among opisthobranchs. A slug that is overlooked expends very little in the way of resources for the very reason that it does not get attacked. Crypsis, however, restricts the animal to those times and places where it is effective and may conflict with a need to forage for food or find a mate. Chemical defense may allow an opisthobranch to survive in more exposed positions, and if very effective the animals may be virtually immune from predators. The metabolites must cost something—how much nobody has yet attempted to estimate. Obtaining them from food must be cheaper than biosynthesizing them *de novo*, but the cost of foraging must be balanced against the savings thus realized.

Chemically defended opisthobranchs are often attacked, as is shown by the frequency with which damaged specimens are found in natural environments. Although they often survive, they would be better off not having been attacked at all. Autotomy may allow an animal to crawl away while the predator feeds upon the autotomized part. The autotomized parts are often dorsal processes that writhe and squirm when detached. Having the dorsal processes contain diverticula of the gut, rather than more nutritious parts of the body such as gonads, is one way of reducing the costs of autotomy. Swimming may allow a slug to escape from benthic predators, and if faced with a high probability of being eaten, exposure to other predators may be a risk well worth taking. Once the predator has been evaded, however, there are a whole new set of problems. The slug may have a hard time locating an appropriate environment.

Defensive anatomy, for instance, that of shells, displays trends that can be followed in the fossil record as well as on a geographical basis (Vermeij, 1978, 1987). Animals are particularly well defended in places where species diversity is high, as in the seas around New Guinea and the Philippines. Although chemical defense had not left a fossil record *per se*, nonetheless the same kinds of trends that characterize shells have been documented in metabolites. Just as tropical shells tend to be more robust, tropical marine animals tend to more toxic (Bakus, 1974, 1981; Coll,

LaBarre, Sammarco, Paul, Williams & Bakus, 1982). This has been well corroborated, though the Antarctic fauna does not fit the trend very well, being a rich source of defensive metabolites (McClintock & Baker, 1997). There are similar trends that go along with the seasons.

The problem of what causes such global patterns, including those of species diversity, body size, the amount of genetical recombination, and all sorts of other things may have no simple answer (Ghiselin, 1974). But they do indicate adaptation, however obscure the causes may be. One interesting study on the seasonal changes in the amount of toxic materials in a sponge shows a low level in the late winter and a higher one in the summer and fall (Turon, Becerro & Uriz, 1996). The kind of naïve reasoning that would relate the difference to temperature as such does not work, for the curve lags far behind. The pattern is rather like that of the number of eggs per clutch in certain birds, which declines during the reproductive season as food becomes more limited and it is more important to protect the young from predators. We say more about such topics at the end of this monograph.

Many of the metabolites that occur in marine animals are pigments, a heterogeneous class of colorful compounds (Bandaranayake, 2006). Some of these are photosynthetic pigments contained in symbiotic algae and cyanobacteria. In other cases they are thought to be sun-screens that prevent damage from ultraviolet light. Others, including alkaloids produced by symbionts, are toxic or distasteful. Still others would appear to have mainly a visual effect, providing for color patterns that may conceal the animals on the one hand (camouflage), or render them conspicuous on the other (warning coloration).

The fact that animals rich in secondary metabolites are often brightly colored is one more piece of evidence in favor of their having a defensive role. They supposedly have a kind of warning coloration that advertises the fact that they are distasteful to the predators. Some of the most conspicuous opisthobranchs in tropical waters are ones that have high levels of metabolites. For instance, dorid nudibranchs of the family Phyllidiidae, such as *Phyllidia ocellata* shown in **Photo 64**, which are well protected by isocyanides and have few natural predators, are generally active during the day and are quite conspicuous (Brunckhorst, 1991). On the other hand it has often been pointed out that many of the colorful opisthobranchs are actually quite inconspicuous when on their food. The mimics of brightly-colored animals may not be as distasteful as their models. Furthermore, the mere fact that an animal is drab and inconspicuous does not necessarily mean that it lacks chemical defense. Opisthobranchs that live among algae and feed on them are often well camouflaged, yet have well-developed chemical defense. Warning coloration is most strongly developed in those opisthobranchs that for one reason or another benefit from being able to venture out into the open with relative impunity. For example, dorid nudibranchs of the genus *Roboastra* pursue other nudibranchs and eat them, thereby deriving both nutriment and defensive metabolites (see Chapter X).

Warning coloration and mimicry are controversial topics that have received a great deal of attention from biologists (for opisthobranchs see the works of Edmunds, 1974, 1987, 1987, 1991; Gosliner & Behrens, 1990; Gosliner, 2001). We will not discuss the issues in detail, but there are some peculiarities of opisthobranchs that make them particularly interesting in this connection. It has been something of a puzzle how natural selection could favor the evolution of conspicuous color patterns in the first place. One might think that a predator interacting with a varied population of prey would tend to notice and to eat the more conspicuous items first. The more conspicuous organisms would be the ones most often to die and would fail to pass their genes on to the next generation. The simplest answer is that often enough the prey escape either uninjured or not mortally so. There is experimental evidence for this (Penney, 2004; Gosliner, 2001). Furthermore, many animals tend to avoid conspicuous prey. Various other ways of getting around that problem have been suggested, but we will only consider one of them (but see Marples, Kelly & Thomas,

2005). This is kin selection, or, if you prefer, familial selection (Harvey, Bull, Pemberton & Paxton, 1982). Basically the idea here is that the unit that gets selected is not an organism, but a family unit made up of close relatives. This would work in such terrestrial animals as butterflies, in which the larvae (caterpillars) hatch out of eggs that are laid in the same place and then hatch out and grow up in close proximity to their siblings. If one of them gets eaten by a predator, and if the predator is repelled, then the remainder of the family will be spared. With most marine gastropods, including most opisthobranchs, these conditions are not met, as was first pointed out by Faulkner and Ghiselin (1988). Tulrot and Sandberg (1991) provided the same argument. It was further developed by Rosenberg (1989, 1991). The usual pattern in opisthobranchs is for the fertilized eggs to be laid in a clutch protected by transparent coverings made up largely of mucopolysaccharide secretion from the genital duct. The embryos remain in the egg mass while they develop. During that period they are in close proximity to their siblings, and therefore the conditions for kin selection are in fact met. Indeed, the eggs of opisthobranchs are often brightly colored and loaded with metabolites as well. By the end of their embryonic development, however, they hatch out as larvae, swim about in the water until they find a suitable place for metamorphosis, and settle on the bottom. Only then do they grow up into mature adults. In consequence, the gene pool gets "stirred up" and the siblings are separated from one another.

It would appear, then, that the kin or familial mechanism of selection will not work. But things are not all that straight forward after all (Cimino & Ghiselin, 2001). Opisthobranchs, although they are simultaneous hermaphrodites, generally do not self-fertilize. They fertilize their eggs with sperm from the prospective father that is stored in a special organ (receptaculum seminis) until the mother fertilizes the eggs. More sperm than are necessary for fertilization are produced, and some of the partner's sperm may be digested in an organ called the gametolytic gland or bursa copulatrix. This allows for a kind of recycling, allowing the sperm-donors to contribute to the next generation but bringing about sperm competition among them. The animals tend to congregate in small groups, and they may copulate repeatedly. Sometimes, as in the case of Notaspidea, they can be found in the field living as pairs. The body of any such animal that has copulated contains not only his and her own germ plasm in the form of unfertilized eggs, but also that of other individuals in the form of viable sperm. Consequently, from a Darwinian point of view a dead opisthobranch is still very much alive, so long as the sperm are capable of engendering offspring.

Perhaps the best way to close this methodological excursus is to emphasize the point that quite a variety of evidence can be brought to bear upon the problems of adaptive significance. Experiments, whether in the field or the laboratory, comparisons whether of behavior or anatomy, and the historical data of biogeography and phylogenetics all provide useful evidence and lend one another mutual support.

Chapter III

Producers and Transformers of Secondary Metabolites

Part 1. Introduction

Although many opisthobranchs biosynthesize defensive metabolites *de novo*, their doing so is a later evolutionary development. Originally they obtained the metabolites from food, and this remains the general rule. Therefore some background material on the organisms from which the metabolites are obtained will be useful in helping us understand the evolution of chemical defense

in opisthobranchs. We shall discuss these organisms in an order that roughly corresponds to their evolutionary relationships and note which metabolites are present in the various taxonomic groups. Naturally we pay most attention to the organisms that play a role in our evolutionary scenario. The branches of interest are treated in an order that puts the gastropods and other mollusks last, thereby providing a smooth transition to the chapters that follow. Before doing that, however, we will briefly consider how the organisms' feeding mechanisms and ways of life relate to how they are defended, whether chemically or otherwise. This provides an ecological, rather than an historical classification. Therefore it often places genealogically unrelated groups together.

Microorganisms include bacteria and unicellular plants and fungi. What they all have in common is, by definition, small size. For that reason many of them can live in small spaces, such as between sand grains or inside other organisms. Organisms that live in close association with other organisms of different species are called "symbionts" and, so far as the interest of the "host" organism goes, these may be innocuous commensals, harmful parasites, or helpful mutualists. Many of the natural products that are extracted from marine animals are widely believed to be produced by symbiotic bacteria or fungi. With a few exceptions, however, the evidence for this is weak. In the case of opisthobranchs there is no evidence for it whatsoever.

Autotrophs are organisms that produce their own food from energy of non-biological origin, mainly by photosynthesis. Most of the supply of energy to the food chain in the sea is provided by unicellular plants that exist as plankton in the water. There is no place for them to hide under such circumstances. Often they are protected by shells or spines, but chemical defense is not unusual. On land the main photosynthetic organisms are macroscopic plants, which live rooted in the soil and for that reason cannot get away from grazing animals. Attached (sessile) plants are also abundant in the sea, but they are mostly restricted to hard bottoms in shallow water. Like terrestrial plants, they cannot run away from grazing animals, and, again like terrestrial plants, they often defend themselves by means of secondary metabolites.

Sessile and sedentary animals are very abundant in the sea. They may live firmly attached to rocks or other hard surfaces. Many burrow in sand or mud. Such animals are heterotrophs, meaning that they depend upon material of biological origin for their energy supply. They feed mainly on material that is suspended in the water or that settles out on the bottom as a deposit. Again, such a life style makes it difficult to run away from grazers or predators, and alternative means of defense are common, including chemical defense.

Many marine animals make their living by slowly crawling about and grazing upon sessile or sedentary animals and plants. The majority of opisthobranchs can be so characterized, as can seastars. They have to be adept at overcoming the defenses of the prey, including the chemical ones. At the same time they are themselves vulnerable to attack by faster-moving predators. One way in which they can defend themselves from such predators is by means of chemical defense. Secondary metabolites derived from food provide a convenient supply.

Chemical defense is less of an advantage to highly motile grazers and predators that can effectively dodge or out-run their attackers or perhaps fight them off. Chemical defense is common among gastropods, though only in those in which the shell is somewhat reduced. In cephalopods, which are active predators that have largely dispensed with a shell, chemical defense is unusual. The little blue-ringed octopus, *Hapalochlaena maculosa*, appears to be an exception. However, this tropical animal kills by means of its venom, which is also used to subdue prey. The venom, tetrodotoxin, evidently of bacterial origin, is contained in the salivary glands (Sheumack, Howden, Spence & Quinn, 1978). Its bite can be fatal to human beings. It has brilliant warning coloration. In general, one expects marine animals that are brightly colored to have an unpleasant taste due to secondary metabolites.

Part 2. Prokaryotes

Prokaryotes are distinguished from eukaryotes on the basis of the structure of the cell. The prokaryote cell is simpler than the eukaryote cell and lacks such features as a nucleus and the membrane that surrounds it. The prokaryotes consist of two major groups of bacteria, Archaebacteria and Eubacteria. The Eukaryotes are more closely related to the Archaebacteria than to Eubacteria: in other words the bacteria are a paraphyletic group. The Eubacteria include all of the bacteria that are of interest in this discussion, and these belong to two closely-related lineages, the Actinobacteria and Cyanobacteria. The Cyanobacteria were formerly considered plants and were called blue-green algae, or Cyanophyta. They form small filaments that are readily visible to the naked eye, and superficially resemble some true algae. A case of poisoning that occurred when people in Hawaii ate the red alga *Gracillaria coronopifolia* was later attributed to the cyanobacteria growing epiphytically on it (Nagai, Yasumoto & Hokama, 1997).

Animals that feed on prokaryotic or eukaryotic "algae" sometimes have switched from one to the other during the course of evolution. This is but one example of how the texture and metabolite content of the food are often more important than its taxonomy. Bacteria are noteworthy for their chemical versatility. Many of them synthesize metabolites that eukaryotes do not. They are particularly important as symbionts that synthesize alkaloids, macrolides, and polyketides. Bacteria that live as symbionts within the bodies of marine animals such as sponges and sea-squirts are the source of alkaloids and some other secondary metabolites. (For review of marine microorganisms and fungi with secondary metabolites, see Pietra, 1997; Bernan, 2001; Engel, Jensen & Fenical, 2002).

Some unfortunate claims have occasionally made, even in print, about the putative role of symbionts in producing secondary metabolites. A particularly glaring example is to be found in a recent publication by Castoe, Stephens, Noonan and Calestani (2006:"2"): "Polyketide compounds, used as defensive mechanisms to deter predation, have been isolated from some marine invertebrates (e.g., sponges and mollusks; Garson, 1989), although these polyketides were subsequently found to be produced by bacterial symbionts (and not encoded in the animal genomes)." What the authors allege simply is not true. They do not cite a single publication in favor of their claim, and had anything of the sort been published it would surely have been brought to our attention. One way or another, what perhaps began as a mere speculation thrown out in casual conversation has become a rumor, or even what is sometimes called an "urban myth." Now, given the fact that some, indeed many, animals make use of metabolites that are produced by their symbionts, it is perfectly reasonable to ask, in any given case, whether it is the host or some guest that synthesizes it. There are two possibilities and we need to apply the proper canons of evidence to decide between them, just as we do when deciding whether metabolites are produced endogenously or are derived from food. Many opisthobranchs obtain metabolites by eating other organisms that in turn get them from symbionts. It has not been difficult to recognize those symbionts, which exist in substantial numbers and are located in particular tissues and specialized organs within the body of the host. In opisthobranchs there are no tissues or organs that are reasonable candidates for harboring such a population of symbionts, and no such populations have been identified. In those cases where biosynthesis has been studied experimentally, and in which the biology of the animals is well understood, the production of metabolites by symbionts, although perhaps not as rigorously excluded as a hostile critic might demand, is highly implausible. In the cephalaspidean *Haminoea*, for example, the animal biosynthesizes defensive metabolites called fusipyrones. But the animals also biosynthesize a series of metabolites called haminols, which are not defensive. Rather they function as alarm pheromones, which alert conspecifics to attack by predators, and probably also

function in mate recognition. Different species have different pheromones (see the section on Cephalaspidea). Our credulity is strained by the notion that the system of haminols would evolve by modification of biosynthetic pathways in the guest rather than in the host. The symbionts are figments of the imagination, in which they function as nothing more than *ad hoc* hypotheses.

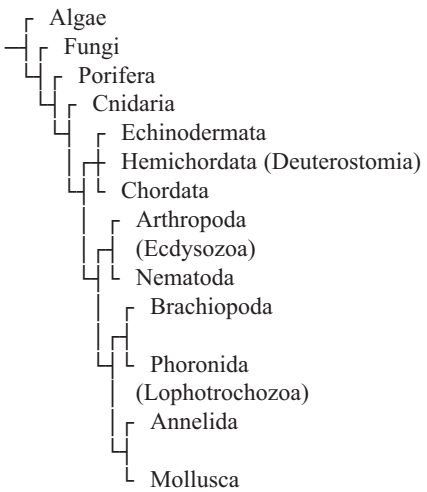
Part 3. Plants and fungi

Except for a few vascular plants such as turtle grass, "marine plants" means algae. Each of the three major groups of algae (Phyophyta, Rhodophyta and Chlorophyta) has given rise to multicellular lineages and each of these is a source of metabolites that are of interest to this discussion. The brown algae (Phyophyta) include the macroscopic sea-weeds called kelps. They are rich in terpenoids, such as the highly toxic dolabellanes (**Atlas 373**) that were first found in the anaspidean opisthobranch *Dolabella* (**Photo 19**). The red algae (Rhodophyta) are currently treated as a division Rhodophycota, with a single class, Rhodophyceae, having two subclasses: Bangiophycideae and Florideophycideae. The Bangiophycideae, which are supposedly more primitive, seem to lack defensive metabolites. On the other hand a wide variety of them have been recorded from the Floridiophycideae. The green algae (Chlorophyta) are the closest relatives of "higher" plants. Green algae of the families Udoteaceae and Caulerpaceae are of particular interest because many opisthobranchs of the order Sacoglossa feed upon them by sucking the sap out of their cells. Probably Udotaceae is paraphyletic, for Caulerpaceae has only one genus, *Caulerpa*.

Terrestrial fungi are familiar to us as yeasts, moulds and mushrooms. They are important sources of antibiotics and other secondary metabolites of medical interest. Marine fungi are usually inconspicuous organisms. A substantial number of fungi that produce secondary metabolites occur as symbionts in sponges (Pietra, 1997; Abrell, Cheng & Crews, 1994; C. Smith et al., 2000; Edrada et al., 2002). Recently quite a number of secondary metabolites have been recovered from fungi that live in association with algae (Abdel-Lateff, König, Fisch, Höller, Jones & Wright, 2002).

Part 4. Animals

The following is a simplified phylogenetic tree of the multicellular animals (Metazoa) with Algae and Fungi shown as outgroups.



Some groups that might have been shown are left out, mainly because they do not play a sig-

nificant role in this discussion. Although the branching sequences shown are debatable, they represent the generally accepted view of things, arrived at by combining the molecular data that have been accumulating over the last few years with the more traditional anatomical and embryological evidence. What sort of animal occupied the first node within the animals, i. e., the (latest) common ancestor, is hard to say, though the monophyly of the group implies that it was multicellular. From that ancestor there are two major lines of descent, the sponges (Porifera) and all the rest. On the basis of some molecular evidence it has been suggested that one lineage of sponges is closer to other animals than to some other sponges. If this be so, then Porifera is paraphyletic and all animals, including ourselves, are modified sponges. The Cnidaria or Coelenterata in the strict sense are such familiar animals as sea-anemones and jellyfishes. Their distinct gut unites them with all the remaining animals, which are called Bilateria, on the basis of their bilateral symmetry. Within the Bilateria there are two very distinct clades that owe their names to the way in which the mouth is formed during embryological development: Deuterostomia and Protostomia.

Deuterostomia consists of three phyla: Echinodermata, Hemichordata, and Chordata. The adults of these three groups are very different, and the connection between them is obvious only when one looks at the early developmental stages. Echinoderms, such as sea-stars and sea-urchins, are here treated as an unresolved trichotomy with Chordata and Hemichordata, as shown on the tree. Chordata includes us vertebrates (Vertebrata), the simple, fish-like creatures called "Amphioxus" (Cephalochordata) and a group of filter-feeders (Urochordata) or tunicates. Among the tunicates, one group (Ascidiacea), the ascidians or sea-squirts consists of sessile animals that are important sources of secondary metabolites.

Protostomia is a much larger group, for it contains several major phyla including Arthropoda, Nematoda, and Mollusca, as well as most of the minor ones. On the basis of molecular data two major clades within the Protostomia have been recognized: Ecdysozoa and Lophotrochozoa. Ecdysozoa derives its name from the characteristic feature of molting the exterior layer of the body (ecdysis) and includes the arthropods (Arthropoda) and nematodes (Nematoda). Lophotrochozoa takes its name from characteristic features of two groups of phyla. The lophophorates (Tentaculata) have a crown of tentacles with which they feed. Three phyla have traditionally been placed in Tentaculata: Phoronida, Ectoprocta (=Bryozoa in the strict sense) and Brachiopoda. Phoronids are tube-dwelling worms. Ectoprocts are colonial creatures sometimes called "moss animals." Brachiopods have bivalved shells, and have a superficial resemblance to mollusks of the class Bivalvia. The Trochozoa are named after the so-called trochophore larva. The segmented worms (Annelida) and the mollusks (Mollusca) are familiar examples, but the group also contains some of their more obscure relatives.

Let us briefly consider an example of how a diagram like this one can be used to address historical questions. The phyla Hemichordata, Phoronida, and Annelida occupy quite distant positions on the tree. Some of these animals live as burrowers in marine mud. Among the hemichordates the Enteropneusta have a wide variety of secondary metabolites that are said to have antimicrobial activity but are believed to play a role in deterring predators. These compounds include bromophenols, halogenated indoles, and ingotin pigments (halogenated alkaloids) (Higa, Fujiyama & Scheuer, 1980; Higa, Ichiba & Okuda, 1985; King, 1986; Higa, Okuda, Steverns, Scheur, He & Clardy, 1987). A burrowing annelid, *Thelepus setosus*, contains a series of five brominated phenolic compounds (Higa & Scheuer, 1974, 1975). And finally, *Phoronopsis viridis*, a tube-dwelling phoronid worm that is abundant on mudflats, contains the halogenated phenolics 2,6-dibromophenol and 2,4,6-tribromophenol (Sheikh & Djerassi, 1975). Given that these animals have such a remote common ancestry, it seems more than likely that they evolved similar metabolites in response to similar environmental circumstances.

Part 5. Animals: Porifera

Sponges have been particularly rich sources of secondary metabolites (Dumdei, Blunt, Munro, Battershill & Page, 1998). They are sessile filter-feeders. Water is taken up from the exterior via numerous small pores that lead into a system of internal channels. The small particles of food that the water contains are extracted by cells that line the chambers. Sponges are loosely integrated and have nothing equivalent to a nervous system. In addition to the soft tissue, there is generally a substantial amount of skeletal material. Although it seems obvious that the skeletal material provides support, to what extent it functions defensively remains an open question (cf. Chanas & Pawlik, 1995; Burns & Ilan, 2003). The skeleton of sponges, when present, generally consists of fibrous protein called spongin, spicules, or both. The bath sponges of commerce are made up of spongin. Spicules are mineral (or occasionally protein) formations. Their structure and chemical composition are important in classification. Additional material, living or not, may be incorporated within a sponge.

Bacteria and fungi can make up a considerable proportion (up to 40%) of a sponge's body, and this is important because these symbionts are sometimes the source of secondary metabolites that get passed up the food-chain (Bewley, Holland & Faulkner, 1996). Most of the metabolites, however, are produced by the sponge, not the symbionts (Faulkner, Harper, Haygood, Salomon & Schmidt, 2000; Salomon, Deerinck, Ellisman & Schmidt, 2001). It seems to be a general rule that the sponges themselves produce terpenoids and their symbionts amino acid derivatives. However, the production of the terpenoids by the sponges has not yet been rigorously confirmed by experiments. In *Dysidea herbacea* (Demospongiae: Dictyoceratida: Dysideidae), the sponge itself produces sesquiterpenoids, whereas its symbiotic cyanobacteria produce amino acids (Unson & Faulkner, 1993). In *Suberea creba* (Demospongiae: Verongida: Aplysinellidae), the sponge produces quinolines, and the symbionts tyrosin metabolites (Faulkner, Harper, Haygood, Salomon & Schmidt, 2000). The bacterial symbionts are transmitted to the next generation via the ova and larvae (Lévi & Lévi, 1976; Sciscioli, Lepore, Gherardi & Scalera-Licali, 1995).

Three extant classes of Porifera have long been recognized: Hexactinellida, Calcarea, and Demospongiae. The three groups are quite distinct, but the relationships among them are uncertain. They differ with respect to the structure and the composition of the skeleton. The mineralized spicules are calcareous in Calcarea, and siliceous in the other two classes. The overwhelming majority of secondary metabolites that have been reported in the literature are from the Demospongiae, which is the most diverse and best studied of the three, and most of the opisthobranchs that obtain their defensive metabolites from sponges get them from this group. However, a few secondary metabolites are known from Calcarea and Hexactinellida. Likewise, as we shall see, a few opisthobranchs feed on Calcarea or Hexactinellida and obtain metabolites from them. Because Porifera is about half a billion years older than Opisthobranchia, it is not an illustration of the two groups coevolving as a unit, but rather of the opisthobranchs taking advantage of different food sources with similar texture and metabolites.

Sponges are hard to classify and a system that reflects the genealogical relationships is definitely work in progress. The community of sponge taxonomists has recently produced a provisional system that has no pretensions to do more than improve upon its predecessors (Hooper & Van Soest, 2002). Therein the families of Demospongiae are arranged in a series of ten orders, most of which roughly correspond to natural groups. The classification definitely tends to put those sponges that have similar metabolites together. We have followed this classification in the text.

Sponges are also hard to identify. That creates serious problems when we try to interpret the literature. The sponges fed upon by opisthobranchs have often been misidentified, sometimes being

placed in the wrong family. Rudman and Bergquist (2007), respectively specialists on the systematics of nudibranchs and sponges, have made a valuable contribution by correcting numerous misidentifications. A number of recent molecular studies provide good evidence that many "species" of sponges are really groups of several cryptic species (Solé-Cava & Thorpe, 1986; Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991; Klatau, Solé-Cava & Borojevic, 1994; Muricy, Boury-Esnault, Bézac & Vacelet, 1996; Muricy, Solé-Cava, Thorpe & Boury-Esnault, 1996; Boury-Esnaut, Klautau, Bézac, Wulff & Solé-Cava, 1999; Nichols & Barnes, 2005; Blanquer & Uriz, 2007). Treating such assemblages as if they were species can have numerous unfortunate consequences. It gives the false impression that certain species are cosmopolitan. It makes it appear that particular species of sponges contain a wider range of metabolites than they actually do. And it distorts our conception of symbiont specificity in the sponges and of feeding specificity of the animals that eat them. *Tedania ignis*, which has been studied extensively from the point of view of metabolites (Schmitz, Gunaserka, Gopichand, Hossain & Van der Helm, 1984), has a close relative, *T. klausi*, from which it differs ecologically (Wulff, 2006). Seastars that eat the former species reject the latter, and angelfish likewise consume *T. klausi* less rapidly.

Part 6. Animals: Cnidaria

The phylum Cnidaria is also known as Coelenterata, although the latter term has sometimes been used for a larger assemblage that also includes the "comb-jellies" (Ctenophora), which may or may not be closely related to them. The group includes such relatively familiar animals as jellyfish, sea-anemones, and various corals and hydroids. Diagnostic of Cnidaria is the presence of cnidae, or stinging capsules. These are organelles that are produced within specialized cells. Upon receiving the appropriate stimulus they release a threadlike process. The cnidae may contain venom and are used both defensively and in capturing and subduing prey. In some cases the venom is powerful enough to kill a human being. Some of the opisthobranchs that eat cnidarians use the cnidae in their own defense (see Chapter XI). Cnidarian life cycles often include two distinct stages, the polyp and the medusa. As a rule, the polyp is a sessile creature that lives as colonies formed by asexual reproduction. The medusa is a jellyfish that, as a general rule again, swims about in the open water. It is noteworthy that the majority of secondary metabolites that are known from cnidarians were found in the polyp stage.

There are three (or in some classifications four) classes of Cnidaria: Anthozoa (anemones and corals), Hydrozoa (hydroids), and Scyphozoa (true jellyfish). It is currently understood that Anthozoa is the sister group of the other two. Lack of a medusa in this group can nonetheless be interpreted as secondary (Scholtz, 2004). Be this as it may, anthozoans are benthic animals with large polyps that often form colonies. The group is subdivided into two subclasses: Hexacorallia and Octocorallia. Hexacorallia includes anemones (order Actiniaria) and the reef-building corals (Scleractinia). There are three orders of Octocorallia (octocorals) with many species that are very rich in secondary metabolites. Two of these orders deserve mention at this time. The gorgonians are called sea-whips and sea-fans. They have a hard skeleton covered with tissue that contains the feeding polyps. This tissue generally contains hard spicules (sclerites) that help to resist the attack of grazers, as well as secondary metabolites. Soft corals are, as the name suggests, soft-bodied, and defensive metabolites are abundant in these as well. Sea pens have a muscular peduncle and live anchored in soft or unconsolidated substrates.

Gerhart (1983) studied the terpenoids of gorgonians from a phylogenetic point of view. He noted that the group can be divided into two lineages. In one branch, consisting of the genera *Gorgonia*, *Pseudopterogorgia*, *Plexaurella*, and *Muricea*, there are sesquiterpenes but not diter-

penes. In the other, consisting of *Briareum*, *Eunicella*, *Eunicea*, and *Pseudoplexaura*, there are diterpenes; sesquiterpenes do occur, but they are limited to *Briareum* and *Eunicella*, which constitute a derived branch within the group. Therefore it seems likely that the defensive use of sesquiterpenes has evolved twice in the gorgonians. Diterpenes also occur in other Octocorallia, but the carbon skeletons are different from those that occur in sponges. In Octocorallia they are cembranolides, whereas in the majority sponges they display the tricyclic spongiane skeleton. Because the mode of biosynthesis is different from that of sponges, and also because there is a different pattern of cyclization, it would seem that they do not go all the way back to the common ancestor of Metazoa (Dai, Garson & Coll, 1991). Another class of metabolites for which the gorgonians are celebrated is the eicosanoids, such as punaglandin-1 (**Atlas 14**), that have already been mentioned. These molecules, however, are widely distributed in animals, where they serve a variety of signaling functions. What is unusual about the gorgonians' eicosanoids is the defensive role that they have come to play.

Part 7. Animals: Deuterostomia (Echinodermata, Hemichordata, and Chordata)

The echinoderms are some of the most structurally and physiologically aberrant organisms in the animal kingdom. Their relationship to other deuterostomes is anything but obvious from adult anatomy. This extreme divergence does not settle the question of their relationships to the other two phyla. They are slow-moving animals, and not surprisingly they are rich in defensive chemicals that have been useful for classification within the phylum. However these metabolites give no hint of their relationship to other animals. They are noteworthy for the presence of saponins, which are steroid and triterpenoid oligosaccharides (Kalinin, Levin & Stonik, 1994). Some plants, but no other animals, have similar metabolites and use them defensively.

Hemichordates, briefly discussed above (p. 212), do not play an important role in our opisthobranch scenario. The chordates are a different matter. One group of these, the subphylum Urochordata, contains a largely sessile group, the sea-squirts or ascidians (Ascidiacea). They live mainly on hard surfaces, and obtain food from the water by filtering it with their gills. Colonies are often formed through budding of the separate "persons" or zooids. As with many other sessile organisms, both physical and chemical defense are well developed. There is a thick, rubbery covering called a tunic, made up largely of β-cellulose. A wide variety of secondary metabolites are present as well. Some of these metabolites are produced by symbiotic algae and bacteria (Kang, Jensen & Fenical, 1996). It seems likely that at least some of the symbionts are transmitted via the eggs and larvae. Some of these metabolites occur in opisthobranchs that feed upon ascidians.

Other metabolites are produced by the ascidians themselves. Some ascidians of the genus *Didemnum* produce eicosanoids, providing an interesting analogy with gorgonians (Lindquist & Fenical, 1989; Niwa, Inagaki & Yamada, 1991). Particularly curious chemicals that are thought to play a defensive role in ascidians are the elemental vanadium and related metals that are produced together with sulphuric acid and unstable hydroquinoid compounds called tunichromes (Stoecker, 1980a, 1980b, 1980c).

Part 8. Animals: Ecdysozoa, Platyhelminthes, Nemertea, Annelida

Ecdysozoa includes the largest phylum in the animal kingdom, Arthropoda, as well as Nematoda and several other phyla that do not interest us here. Arthropods are numerically dominant on the land because there are so many insects, and in the sea because there are so many crustaceans. Marine ecdysozoans, including crustaceans, do have some chemical defense but it has had little if any impact on the opisthobranchs. The same may be said of flatworms (Platyhelminthes),

and nemerteans or ribbon worms (Nemertea). Some annelids or segmented worms (Annelida) have defensive metabolites. Poisonous annelids are eaten by one very obscure group of opisthobranchs, cephalaspideans related to *Acteon* and *Hydatina*, but data on their secondary metabolites are known for only one of these, *Micromelo undata*, and its polypropionates (**Atlas 152, 153**) are probably biosynthesized *de novo*, not derived from food.

Part 9. Animals: Ectoprocta and Mollusca

The Ectoprocta, or Bryozoa in the strict sense, are popularly known as "moss animals" because the branching colonies may resemble moss. They are sessile, colonial animals that feed on particles in the water by means of a crown of tentacles. The small zooids that bear the tentacles are generally protected by some kind of external covering. This is in addition to chemical defense (reviews: Christophersen, 1985; Sharp, Winston & Porter, 2007). Ectoprocts are rich in alkaloids and macrocyclic polyethers, and for both of these compounds a bacterial origin has been claimed (Anthoni, Nielsen, Pereira & Christophersen, 1990). Ectoprocts brood the developing embryos, and this evidently facilitates the transfer of symbionts from one generation to the next. Woollacott (1981) found, in the larvae of some ectoprocts, bacteria that are suspected of producing macrocyclic polyethers called bryostatins (review in Newman, 2005). Formerly it was thought that the ectoproct *Bugula neritina* contains a variety of bryostatins. It turns out, however, that *Bugula neritina* is not a single species, but rather a complex of several cryptic ones, which have different bacterial symbionts (Davidson & Haygood, 1999).

Mollusks for the most part are slow-moving animals that are defended from predators by a strong shell. Bivalves of the genus *Pinna* live attached to the bottom in the tropics and their shell is not very strong. They have been found to contain the polyether alkaloid pinnatoxin (**Atlas 62**) (Chou, Haino, Kuramoto & Uemura, 1996). Some other bivalves contain secondary metabolites derived from food that render them poisonous to human beings and other animals. A shell has been dispensed with in the fast-moving cephalopods such as squid, which are adept at evading predators. The powerful beaks that cephalopods use in subduing prey and dealing with predators are supplemented by venomous secretions.

Chapter IV

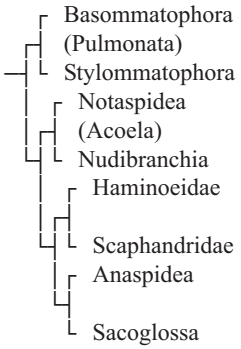
Introduction to Gastropod Diversity and Systematics

The gastropod common ancestor probably was herbivorous, or perhaps omnivorous with a substantial amount of plant material in its diet. The radula allowed it to scrape up algal materials, which were then swallowed and digested. Numerous lineages subsequently switched to other kinds of food, although many remained herbivorous. In other words, the broad picture of gastropod evolution has been an adaptive radiation based on taking advantage of a diverse food supply. It is perhaps better to view the gastropods as "entrepreneurs" rather than as avoiders of competition.

Reiterating what has been said about their higher taxonomy, gastropods have been subdivided into three subclasses: Prosobranchia, Opisthobranchia, and Pulmonata. Pulmonates and opisthobranchs are modified prosobranchs, and Prosobranchia is a paraphyletic grade that represents what is left over after Opisthobranchia and Pulmonata have been removed. The pulmonates and opisthobranchs form a group called Euthyneura, and share a common ancestry with some of the more advanced prosobranchs called mesogastropods. This group forms a grade in an old fashioned clas-

sification that goes back to Johannes Thiele (1931), who divided Prosobranchia into the orders Archaeogastropoda, Mesogastropoda and Neogastropoda. In modern classifications Euthyneura is placed with some poorly-understood but evidently related forms in a group called Heterogastropoda.

Contemporary systematists accept the monophyly of Pulmonata and Euthyneura, but there is much uncertainty as to where the various groups of opisthobranchs and their somewhat more remote relatives fit in. It is possible that some groups of opisthobranchs are genealogically closer to pulmonates than to other opisthobranchs. There are some minor groups that probably belong somewhere close to the opisthobranchs but exactly where is uncertain. For the present discussion what really matters is that one order of opisthobranchs, Cephalaspidea, evidently gave rise to all the other orders and perhaps to Pulmonata as well. At present approximately eight such orders are recognized, and evidently most of them arose from different lineages within the Cephalaspidea. In other words, Cephalaspidea is a highly paraphyletic group. The following tree shows Pulmonata as the sister group of Opisthobranchia with a lineage consisting of two orders, Notaspidea and Nudibranchia forming a first branch, followed by two families of the order Cephalaspidea and finally a branch consisting of the orders Anaspidea and Sacoglossa.



Before discussing the opisthobranchs and pulmonates let us provide a little information about those prosobranchs that are of interest from the point of view of chemical defense. One of these is a group of snails, the Lamellariidae, that feed upon tunicates and use the alkaloids from them defensively (Andersen, Faulkner, He, Van Duyne & Clardy, 1985; Carroll & Scheuer, 1990; McClintock, Baker, Hamann, Yoshida, Slattery, Heire, Bryan, Joyablake & Moon, 1994; Kitahara, Nakahara, Yonezwa, Nagatsu, Shibano & Kubo 1997). In some of these animals the shell is considerably reduced and overgrown by the mantle, giving them a superficial resemblance to nudibranchs, for which they are often mistaken. They can be remarkably well camouflaged when resting on colonies of tunicates (Ghiselin, 1964). Juveniles of the cowrie *Ovula ovum* mimic the distasteful dorid nudibranchs *Phyllidia varicosa* and *P. elegans* (Gosliner & Behrens, 1990; E. Mollo, personal communication). **Photo 60** shows the cowrie with its expanded mantle above a detail of the integument of the nudibranch.

Limpets, like slugs, are not a natural group of mollusks. They are a polyphyletic assemblage of snails in which the shell has become cap-shaped, covering the animal's dorsal surface. This configuration means that the snail can no longer withdraw within its shell and close the opening with an operculum, but it can protect itself by clinging to a firm substrate. Limpets have evolved from coiled snails several times in Prosobranchia, Opisthobranchia, and Pulmonata. Among prosobranchs there is one group, Patellogastropoda, in which an intertidal limpet, *Lottia limatula* (formerly *Acmaea limatula*, then *Collisella limatula*), supplements mechanical defense with chemical defense. It contains triterpenoids such as limatulone (**Atlas 517**) (Albizati, Pawlik & Faulkner,

1985; Mori, Takikawa, Kido Albizati, & Faulkner, 1992; Pawlik, Albizati & Faulkner, 1986). Superficially similar triterpenoids, testudinariols A and B (**Atlas 515, 516**), occur in an opisthobranch, *Pleurobranchus testudinarius* (**Photo 43**) (Spinella, Mollo, Trivellone & Cimino, 1997). Testudinariol B also occurs in an as yet unidentified species of *Pleurobranchus* from China (Carbone, 2007). The metabolites in both the prosobranch and the opisthobranchs exhibit a squalane skeleton that apparently is a dimer, formed by the union of a pair of farnesyl sesquiterpenoid moieties.

We begin our account of Euthyneura by an effort to reconstruct the features on the common ancestor of opisthobranchs and pulmonates. This has been attempted by several authors, mainly on the basis of comparing them to more distantly related animals and trying to decide what the primitive conditions are on a physiological basis (Ghiselin, 1966b; Gosliner, 1981). One criterion that needs to be avoided is that of treating such "primitive" animals as the cephalaspidean opisthobranch *Acteon*, and especially its best-known representative, *A. tornatilis*, as if it were the common ancestor. Although primitive in many respects, such as having a rather well developed shell and less of the detorsion that is characteristic of opisthobranchs, *Acteon* has some derived characters, most notably a carnivorous diet and a reproductive system with a separate vas deferens instead of an open, ciliated groove. Presence (primitive) or absence (derived) of an operculum in the adult varies within the family Acteonidae. *A. tornatilis* has a much modified radula, assuming that what has been called a radula is not something else (Gabe & Prenant, 1953).

The common ancestor had a shell, well enough developed that the animal could withdraw into it, supplemented by an operculum. The nervous system did not display many of the effects of detorsion and concentration of ganglia that characterize the more derived representatives of the group. The animal fed mainly on plant material, perhaps including some detritus. It had a relatively unspecialized radula, and its gut possessed at most some cuticularizations rather than the gizzard plates that characterize some of the more derived forms. We may speculate that some reduction of the shell had already begun, perhaps as a consequence of feeding on chemically protected food organisms. A benthic habitat with a significant amount of sediment can be justified on the basis of the changes in the gills and their surrounding (mantle) cavity that can be documented in both opisthobranchs and pulmonates. The mechanisms causing movement of water through the cavity and over the gill have changed, and in correlation so too has the structure of the gill. Instead of the respiratory current being produced mainly by cilia that cover the gill surface, water is moved by the action of bands of cilia behind the gill, and the gill itself presents a series of folds, or plicae, rather than comb-like structures to the water that flows over it (in other words there was a plicate rather than a pectinate gill).

The ancestor was a simultaneous hermaphrodite with an undivided gonoduct, organs that stored and processed spermatozoa, and a series of regions that deposited three layers of material (albumen, membrane, and mucus) around them to form an egg mass. From the common opening there was a ciliated groove that conveyed sperm to the penis, which was located at the anterior end of the body. The life cycle included a free-swimming larva that emerged from a protective egg mass.

Some authors have advocated alternative views about the ancestral state of the gills and the male portion of the reproductive system. So far as the gills go, the "evidence" is merely that the gills are different, not that there is anything implausible about the derivation. The claim that the male part of the system has evolved from a closed duct into an open groove is obviously an attempt to justify physiologically implausible results instead of asking what went wrong with the analysis.

Molecular as well as anatomical evidence indicates that both Opisthobranchia and Pulmonata are monophyletic lineages. The possibility that one opisthobranch lineage or another is closer to

the pulmonates or branched off earlier cannot be ruled out, however It seems likely, then, that the pulmonate stock branched off before the origin of the head-shield that is so conspicuous in many cephalaspideans, and from which they take their name.

In the pulmonates, the mantle cavity has been converted into a lung. Arguments against this homology have been firmly and effectually refuted by Ruthensteiner (1997). One lineage of Pulmonata, Stylommatophora, derives its name from the stalked eyes that are so conspicuous in garden snails. Stylommatophorans are remarkably successful as terrestrial animals. Another lineage, Basommatophora, is named on the basis of such stalks not being present. Basommatophorans are largely fresh-water animals, though a few — the ones that interest us — remain marine. There are a few pulmonates of uncertain relationships, including some marine forms with stalked eyes, such as *Onchidium*, which likewise interest us. Because there are only two pulmonate lineages to deal with, all we need to know is that they are distinct, and therefore we will not go further into pulmonate relationships.

The notion that marine pulmonates are secondarily so is a myth, easily refuted by the fact that after hatching out of their egg masses they swim about as "veliger" larvae like those of many other marine gastropods, including opisthobranchs. Marine pulmonates flourish as intertidal animals. They benefit from the ability to breathe and remain active both when the tide is in and when the tide is out, generally obtaining food by grazing on small plants that live on the surface of rocks, plants and animals.

The marine Basommatophora that interest us are all intertidal limpets. The genus *Siphonaria* (reviewed by Hodgson, 1999) has been extensively studied because it contains polypropionates (Hochlowski & Faulkner, 1983, 1984; Capon & Faulkner, 1984; Hochlowski, Faulkner, Matsumoto & Clardy, 1983; Hochlowski, Coll, Faulkner, Biskupiak, Ireland, Zheng, He & Clardy, 1984; Manker & Faulkner, 1986, 1989a, 1989b; Arimoto, Yokoyama, Nakamura, Okumura & Uemura, 1996; Norte, Cataldo & Gonzáles, 1988). The polypropionates are quite diverse, even when laboratory artifacts are discounted. These animals graze upon the material that covers intertidal surfaces and we may assume that they are basically herbivorous. The absence of polypropionates in the gut suggests that they are not of dietary origin. Their location in the mucus and around the rim of the mantle is what one would expect if they are in fact defensive. Hochlowski, Faulkner, Matsumoto and Clardy (1983) found that they are toxic to fish.

Darias, Cueto and Díaz-Marrero (2006) divided the propionates of *Siphonaria* into two distinct classes, I and II. The class I propionates, such as siphonaienolone (**Atlas 161**), are essentially acyclic and have a linear chain, and differ from one another mainly in the length of the chain. They are similar in their structure to polypropionates that occur in opisthobranchs of the order Cephalaspidea (see below). The class II polypropionates, such as siphonarin A (**Atlas 160**), are much more complicated and variable and bear considerable resemblance to compounds that are known from actinomycetes. But they also have some structural analogies with propionates that occur in pulmonates of the family Onchidiidae, to be discussed later in this chapter. The same as well as other authors have made some vague remarks about common origins, but it is far from clear what these are supposed to be. It is possible that the similarities represent common ancestral conditions, in other words, plesiomorphies. Although the cephalaspideans and marine pulmonates are on separate branches there is no reason why they could not have retained some primitive characters. Alternatively, there could have been a certain amount of evolutionary parallelism or convergence. Until the sources of these metabolites have been studied there will probably be no clear solution to this problem.

Basommatophorans of the genus *Trimusculus* feed with a mucous net, with which they capture plankton (Rice, 1985). Polypropionates have not been found in this genus, and it is not because of

failure to look for them. The four species that have been studied contain unique diterpenoids (**Atlas 460**) (Manker & Faulkner, 1987, 1996; Díaz-Marrero, Dorta, Cueto, Rovirosa, San-Martín, Loyola & Darias, 2003; Darias, Cueto & Díaz-Marrero, 2006; Van Wyk, Gray, Whibley, Osoniyi & Hendricks, 2008). They are very similar to some metabolites (**Atlas 351, 352**) found in the notaspidean *Pleurobranchaea meckelii* (see below). Although these limpets are effective in defending themselves from attacks by sea-stars, those predators that ingest the limpets seem not to be harmed (Rice, 1985). There is no clear answer to the question of whether *Trimusculus* never evolved propionates or whether it changed from propionates to terpenoids as defensive metabolites. Given, however, the fact that the defensive use of polypropionates has evolved repeatedly in euthyneurous gastropods, the former hypothesis is at least plausible.

The other group of marine pulmonates of interest to us is the family Onchidiidae, in which a shell is entirely lacking in the adults. Their stalked eyes suggest that they should be placed in the Stylommatophora together with terrestrial snails and slugs, but it is common practice to put them in a separate group. There is no gill, and they breathe air through an opening at the posterior end of the body. Some respiratory exchange also occurs via the general surface of the body. The dorsum is somewhat leathery, and there are repugnatorial glands that contain defensive metabolites. These metabolites include sesquiterpenoids, depsipeptide acetates, and propionates. In *Onchidella binneyi*, the secretion consists largely of the enol acetate sesquiterpenoid onchidal (**Atlas 216**) (Ireland & Faulkner, 1978). This is the protected form of the molecule. When the animal is attacked, onchidal is changed into the active form, ancistrodial (**Atlas 217**), which has the common dialdehyde structure with an adjacent double bond. Ancistrodial is also present in the defensive secretion of a termite (Baker, Briner & Evans, 1978).

Abramson, Radic, Manker, Faulkner and Taylor (1989) found geographical differences in the concentration of onchidal from different species in different places. *Onchidella binneyi* from Baja California had 230 micrograms per animal, whereas *O. borealis* from California had only 33 micrograms per animal, showing the usual pattern of more chemical defense in lower latitudes. Nonetheless, the repugnatorial gland secretion of *O. borealis* is strikingly effective in deterring attacks by seastars, and the slugs are largely left alone by predators (Young, Greenwood & Powell, 1986). *Peronia peroni* has polypropionates called peronatriols (**Atlas 158**). These animals are eaten by human beings, but evidently only after the dorsal surface has been cleaned off (Biskupiak & Ireland, 1985).

A cytotoxic depsipeptide called onchidin (**Atlas 675**) has been recorded from an unidentified tropical species of the genus *Onchidium* (Rodíguez, Fernández, Quiñoá, Riguera, Debitus & Pouchet, 1994). A similar depsipeptide, onchidin B, occurs in a likewise unidentified *Onchidium* (Fernández, Rodríguez, Quiñoá, Riguera, Muñoz, Fernández-Suárez & Debitus, 1996). The same group of investigators found cytotoxic acetates and propionates in another unidentified tropical *Onchidium* species (Rodíguez, Riguera & Debitus, 1992). Ilikonapyrone (**Atlas 151**) esters have been reported from *Onchidium verrucatum* (Ireland, Biskupiak, Hite, Rapposch, Scheuer & Ruble, 1984).

In pulmonates, then, polypropionates are widespread, but other metabolites occur in addition to, or instead of, them in what would seem to be the more modified representatives of the group. This pattern is rather difficult to interpret historically. One possibility is that initially the marine pulmonates were defended chemically by some other metabolite and began to secrete polypropionates when they shifted to the intertidal habitat. The substitution of diterpenoids for polypropionates in *Trimusculus* would then be a secondary shift, resulting from the availability of new metabolites when a new feeding mechanism evolved. In *Onchidium* and its relatives an analogous change might have led to additional compounds being added to the secretion of the repugnatorial

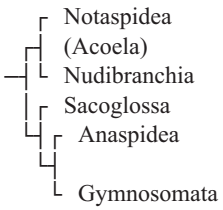
glands. It may be, however, that the defensive use of polypropionates goes back to the common ancestor of pulmonates and opisthobranchs.

Chapter V

Opisthobranch Systematics and Phylogeny

Traditionally, the opisthobranchs have been treated as a sub-class divided into approximately eight orders, namely: Cephalaspidea, Anaspidea, Sacoglossa, Notaspidea, Nudibranchia, Acochlidiacea, Thecosomata, and Gymnosomata. Let us briefly characterize these groups, preparatory to a discussion of their genealogy.

The following diagram indicates likely relationships of five of them, leaving out the paraphyletic Cephalaspidea and the groups for which there are no data on chemical defense:



The Cephalaspidea derive their name from the head-shield at the front of the body. This structure is conspicuous in the specimen of *Bulla striata* shown with its eggs in **Photo 9**. They are also called Bullomorpha by allusion to the genus *Bulla*. The vernacular name, "bubble-shell," alludes to the fragile shells of many cephalaspideans. The shell may be well developed, but all stages of reduction occur. Cephalaspidea is considered the ancestral stock from which the other opisthobranch groups, or at least some of them, have separately evolved. The group as traditionally conceived, however, is a paraphyletic grade. To make it monophyletic it would be necessary to remove some lineages from this group, and taking this step recently has been suggested by Malaquias, Mackenzie-Dodds, Gosliner, Bouchet and Reid (2009).

The Anaspidea derive their name from the secondary modification of the head shield. They are also called Aplysiomorpha by reference to the familiar genus *Aplysia*, which has been a favorite experimental subject for neurophysiology (**Photos 16 & 18**). The head shield has been modified so that there are two pairs of rolled projections called "rhinophores" that resemble ears. These have a sensory function. One genus, *Akera*, however, still retains the unmodified head shield and fragile shell, making it a good transitional form connecting the group with Cephalaspidea (**Photo 17**). Otherwise the body has expanded considerably and the shell is proportionally reduced.

The Sacoglossa, or Ascoglossa, are named on the basis of the sac, or ascus, that holds used teeth. *Lobiger serradifalci* (**Photo 28**) and *Calyphilla mediterranea* (**Photo 40**) are good examples. There is no vernacular name, but they are sometimes called "sap-sucking slugs" from the way they feed upon algae by piercing the cell with their highly modified radular teeth, and then sucking in the contents. Typically they have a single pair of rolled rhinophores, resembling those of anaspideans, but the retention of a head shield in more primitive representatives of both groups shows that this is a result of evolutionary parallelism or convergence. Some sacoglossans again have shells like those of cephalaspideans, and old-fashioned systematists classified them as such. In one lineage, the shell has been modified so that it looks like the bilaterally divided shell of bivalves

rather than the coiled shell of gastropods. But complete loss of the shell in the adults is the general rule.

The Notaspidea have a "shield" on the dorsal surface that is not equivalent to the head shield of other opisthobranchs. *Pleurobranchaea meckelii*, shown with its eggs in **Photo 45**, is a good example. The shell is considerably reduced in all notaspideans, and the mantle cavity has virtually disappeared, so that it no longer covers the gill, which lies along the right side of the animal's body. This configuration led to the coinage of a rather ludicrous vernacular name "side-gilled slug," but "pleurobranch" seems a more sensible term. Another name for the group is Pleurobranchomorpha, after the genus *Pleurobranchus*. Recent phylogenetic studies have suggested that some of the animals traditionally treated as the most basal notaspideans are related to other lineages of opisthobranchs. A group Nudipleura would therefore consist of the remaining notaspideans and their closest relatives, the nudibranchs.

The Nudibranchia, or nudibranchs, derive their name from the naked gills. Gills, when present, may occur as a cluster near the posterior end of the body as they do in such dorid nudibranchs as *Hypselodoris nigrostriata* (**Photo 93**). Or they may take the form of elongate projections of the dorsal surface of the body (cerata), as in *Godiva quadricolor*, shown with its eggs in **Photo 126**. The head almost always bears rhinophores. The shell is absent, except in the larvae.

Acochlidiacea is the name for an obscure group of sand-dwelling opisthobranchs that includes the genus *Acochlidium*. Because these animals have not been studied from the point of view of chemical defense we need only mention them.

The last two orders are called "pteropods" on the basis of the wing-like projections of the foot, with which they swim about in the plankton. They were long considered a polyphyletic group, but an increasing amount of evidence seems to indicate that they are a monophyletic assemblage closely related to Anaspidea (see p. 240).

Thecosomata, the thecosomes, or thecosomatous pteropods, usually have their bodies protected by a shell or some other firm covering. They are herbivores that feed on phytoplankton by means of mucous nets. Chemical defense has not yet been recorded from this group.

Gymnosomata, the gymnosomes, or gymnosomatous pteropods, have naked bodies; in other words they have no shell as adults. They are carnivores, often feeding on thecosomes. Chemical defense is known in only one species.

Students of opisthobranch phylogenetics have had to deal with a situation in which snails have repeatedly evolved into slugs. In some cases, it has been a fairly straightforward task to show, for example, that the resemblances between nudibranchs and shell-less sacoglossans are superficial (Russell, 1929). Efforts to arrange opisthobranchs into series of grades, from primitive to advanced (Boettger, 1954), have always failed. However, subsequent efforts to infer branching sequences have suffered somewhat from the use of characters that are apt to evolve repeatedly in separate lineages, and from using only a very limited sample of the available evidence. The morphological trees recently published by Mikkelsen (1996, 2002) and others do not give a consistent picture of the relationships, and the molecular trees (Wägele, Vonnemann & Wägele 2003; Vonnemann, Schrödl, Klussmann-Kolb & Wägele, 2005) leave some of the more interesting relationships undetermined. An effort to use sequences of histone H3 genes to determine deeper relationships within the Euthyneura gave disappointing results (Dinapoli, Tamer, Franssen, Waduvilozhath & Klussmann-Kolb, 2007). Exact branching sequences are notoriously difficult to obtain. Some groups, such as the pleurobranch genus *Tylodina*, move all over the place, depending on which lines of evidence are utilized. Nonetheless, many relationships are well enough supported by these studies that we can utilize them for our purposes. Research based on a larger and more diverse data base will probably give much better trees than those that have been produced by formal cladistic

analyses thus far. For part of the tree we already have that. Meanwhile it will be necessary to make do with the best available evidence.

Whatever the evidence that is used, it is obvious that Cephalaspidea in the traditional sense (Cephalaspidea *sensu lato*) is a paraphyletic grade, from which several of the other orders have evolved. The problem then becomes one of associating the various cephalaspidean lineages with the derived orders, and with one another. There is good evidence for treating some of the orders as sister groups. One of these is Notaspidea + Nudibranchia. This group was called "Acoela" by Thiele (1931); more recent authors have introduced various synonyms. The morphological evidence is reasonably consistent with the molecular trees. To that may be added the chromosome numbers. The pleurobranch *Tylodina*, as mentioned above, is problematic in the extreme. Its chromosome number is unknown. Beyond the naturalness of Acoela, however, we cannot say very much. It does not show a clear relationship to any other lineage of opisthobranchs. It may be the first lineage of opisthobranchs to have branched off after the separation of the pulmonates. That does not matter very much for the present analysis however. Another clade that may be recognized consists of Anaspidea + Sacoglossa. It is reasonably consistent with the published molecular and morphological trees, and additional evidence, often overlooked, supports it as well. We will discuss that in the appropriate place (pp. 228-229).

Diversification within the Opisthobranchia has been marked by the evolution of more specialized herbivores on the one hand and shifts to various kinds of animals as food on the other. That same pattern may be observed within the Cephalaspidea. In many cephalaspideans there are well-developed gizzard plates used in processing the food, but others have little more than a cuticle in the same location. Therefore it does not seem likely that there were well-developed gizzard plates in the ancestral opisthobranch or the ancestral cephalaspidean. With shifts to a soft diet, the plates often become lost, so that their absence is not necessarily the original condition.

Turning now to cephalaspidean relationships, it is much easier to establish a few groups of close relatives on the basis of derived characters than it is to relate those groups to one another. Traditional classifications generally list a series of families, with familial rank being largely a euphemism for uncertainty as to relationship. We will put these together in three groups, the relationships among which are problematic.

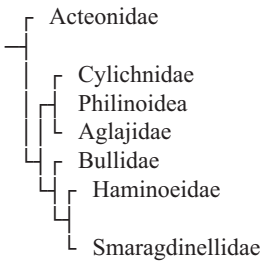
To begin, we have four families that are related to *Acteon*: Acteonidae, Bullinidae, Hydatinidae and Ringiculidae. Leaving the last of these out of consideration, three main points need to be stressed. First, although *Acteon* is not the living fossil it is sometimes considered, in most phylogenetic analyses it comes out in a basal position and often is associated with other clades. Second, *Acteon* shares derived characters of the reproductive system with *Hydatina* (Hydatinidae) and its relatives. And third, the Acteonidae and Hydatinidae are no longer herbivorous, but have switched to feeding upon annelids, which are soft-bodied worms. Therefore gizzard plates would be of no advantage to these animals, and given the switch in diet the absence of such plates could be secondary. On the basis of reproductive anatomy Ghiselin (1966b) suggested a possible relationship between the Acteonidae and Hydatinidae on the one hand and the Notaspidea and Nudibranchia on the other. This relationship has been supported by molecular (rRNA) evidence (Vonnemann, Schrödl, Klussmann-Kolb & Wägele, 2005). This puts two groups lacking a gizzard together and it may be a primitive trait. The Ringiculidae, with the genus *Ringicula*, are of uncertain relationships and have not yet been studied from the point of view of chemical defense. They do, however, have repugnatorial glands on the cephalic shield. The presence in this genus of a (primitive) monaulic (undivided) reproductive system implies that they are the sister group of the rest (Gosliner, 1981). On the basis of new results, Malaquias, Mackenzie-Dobbs, Gosliner and Reid (2009) have advocated recognizing a separate taxon for this lineage, and placing most of the other

cephalaspideans in Cephalaspidea *sensu strictu*.

Next, there are several families that (evidently) retain the herbivorous diet: Bullidae, Haminoeidae, and Runcinidae, and perhaps Diaphanidae and Notodiaphanidae (which are not closely related to each other) as well. Gizzard plates are present in the Bullidae, Haminoeidae and Runcinidae, and the latter two families can be united by the structure and manner of functioning of the gizzard plates, which rock against each other from anterior to posterior and shred the algae or, in the case of a small group within *Haminoea*, cyanobacteria, upon which the animals feed. The resemblance is highly detailed, not, as Mikkelsen (2002:98) says, superficial, although there are four plates, not three. Such characters as the presence of a seminal bulb that forms spermatophores are possible synapomorphies connecting them with Cephalaspidea *sensu strictu* However, molecular studies seem to indicate that the family Runcinidae, although monophyletic, is not closely related to other cephalaspideans (Malaquias, Mackenzie-Dobbs, Gosliner & Reid, 2009). The trouble is that these studies do not indicate the relationship of Runcinidae to any other lineage. In the Bullidae the plates are less elaborate and crush rather than shred the food, as do those of other groups to be discussed later. This herbivorous assemblage is probably a paraphyletic grade rather than a single lineage. However, it does contain some well-supported monophyletic groups, most notably one consisting of *Atys, Haminoea, Phanerophthalmus*, and *Smaragdinella* (see Malaquias, Mackenzie-Dobbs, Gosliner & Reid, 2009), for which we have a considerable amount of data on chemical defense.

And finally, there are several families of carnivorous opisthobranchs in which there are gizzard plates, or in which there is good reason to believe that such plates have been lost: Retusidae, Scaphandridae, Philinidae, Aglajidae, Gastropteridae and Philinoglossidae. These plates are usually three in number, not counting any accessory ones that may be present. They are used in crushing shelled prey, such as mollusks and even foraminiferans. The plates squeeze together due to the action of muscles, and in the most extremely developed forms they act rather like a nutcracker. Shells are well developed in some members of the group but these tend to become much reduced. When there has been a shift towards eating somewhat more motile prey items that are not well defended by shells, such as opisthobranchs, the gizzard and shell both become reduced. It seems likely that this is a polyphyletic assemblage, reflecting a shift to carnivory. But there is good evidence for a monophyletic group that consists of *Philine, Navanax, Aglaja*, and related genera. Again, there are data on chemical defense for this assemblage.

The following tree corresponds to the best estimate we can give at this time of the relationships among the cephalaspideans with chemical defense:



The Acteonidae and allies are treated as the first branch to have diverged from the cephalaspidean stock. There is evidence that these are the closest relatives of nudibranchs and notaspideans. A second lineage of cephalaspideans, herbivorous and without gizzard plates, gave rise to the remaining opisthobranchs, including Cephalaspidea *sensu strictu*, the first branch of which, according to Malaquias, Mackenzie-Dobbs, Gosliner and Reid (2009) consists of the genus

Diaphana without gizzard plates and supposedly primitively so. The remaining Cephalaspidea *s.s.* are the herbivorous forms such as Bullidae and Haminoeidae, together with carnivorous lineages derived from herbivores, primitively with gizzard plates. No other orders derived from this assemblage.

Chapter VI

Cephalaspidea

We are now in a position to discuss the evolution of chemical defense within the (paraphyletic) assemblage Cephalaspidea *sensu lato*, or, in other words, Architectibranchia and Cephalaspidea *sensu strictu*. Details of the classification of other orders will be provided later. Given the common ancestry of pulmonates and opisthobranchs it seems clear that the ancestral cephalaspidean was herbivorous and also that it already possessed some kind of chemical defense. On the other hand, exactly what it fed on, what metabolites it might have derived from that source, and whether it used polypropionates or something else are questions that we cannot answer at this time.

Acteon and its allies have been little studied from the point of view of chemical defense. The group as a whole appears to consist of animals that feed upon annelids, some of which are toxic. *Acteon* has a well developed shell, as do its close relatives such as the Oregonian *Rictaxis punctocaelatus*, shown in **Photo 2**. However, in the tropical genera *Hydatina* and *Micromelo*, shown in **Photos 3** and **4**, the shell is fragile, considerably reduced, and colorful. Polypropionates (Micromelones A and B) (**Atlas 152, 153**) that may have a defensive function have lately been described from *Micromelo undata* (Napolitano, Souto, Fernández & Norte, 2008). Such noncontiguous polypropionates are most unusual, although some have been found in Notaspidea (see Chapter IX).

Chemical defense is widespread in the herbivorous group that includes *Bulla*, *Haminoea,* and *Smaragdinella. Bulla* itself is a generalist herbivore with a relatively primitive gizzard. Polypropionates that resemble those of pulmonates have been found in two species of this genus. The Mediterranean *Bulla striata* (**Photo 9**) produces a series of polypropionates called aglajnes (**Atlas 114-116**). These metabolites were first detected in a cephalaspidean of the family Aglajidae, *Philinopsis depicta* (formerly *Aglaja*) (**Photo 12**) (Cimino, Sodano & Spinella, 1987), which feeds upon *Bulla* and uses the polypropionates defensively. The polypropionates are located in a series of white glands along the margin of the mantle (Marin, Alvarez, Cimino & Spinella, 1999). The Californian *Bulla gouldiana* contains a similar series of polypropionates that get transferred to another cephalaspidean of the family Aglajidae, *Navanax inermis* (**Photo 13**). Spinella, Alvarez and Cimino (1993) did bioassays of niuhinone-B (**Atlas 113**), isopulo'upone (**Atlas 70**) and 5,6-dehydroaglajne-3 (**Atlas 117**). The first of these compounds was not toxic to mosquito fish, but it was toxic to brine shrimp. The latter two were very toxic to both mosquito fish and brine shrimp.

The family Haminoeidae contains a large number of species of small animals with delicate and often transparent shells. A wealth of secondary chemicals have been described from them, but many forms have not yet been investigated. From *Haminoea cymbalum* (**Photo 8**), Poiner, Paul, and Scheuer (1989) recovered a halogenated polyacetate, kumepaloxane (**Atlas 204**). This metabolite, which is exuded in mucus when the animal is molested, deters feeding by fish. It is unusual, but related metabolites have been found in the red alga *Laurencia* and in a sponge, *Haliclona* (Demospongiae: Haplosclerida: Haplosclerina: Chalinidae) (**Atlas 205**) (Capon, Ghisalberti & Jefferies, 1982). The same compound was later found in *Haminoea cymbalum* from India (Fontana, Ciavatta, D'Souza, Mollo, Naik, Paarameswaran, Wahidula & Cimino, 2001) and in the

Mediterranean *Haminoea cyanomarginata* (**Photo 7**) (Carbone, 2007; Mollo, Gavagnin, Carbone, Castelluccio, Pozone, Roussis, Templado, Ghiselin & Cimino, 2008). Such a compound, which is biogenetically related to a typical *Laurencia* sesquiterpenoid, obtusenal, is very unusual in sponges (Capon, Ghisalberti & Jefferies, 1982). Both of these cephalaspidean species are brightly colored and conspicuous, suggesting warning coloration. *Haminoea templadoi* contains an unusual fatty acid, 10,15-eicosadienoic acid (**Atlas 2**) (Carballeira, Anastacio, Salvà & Ortega, 1992). It has not been implicated in chemical defense.

A series of 3-alkylpyridine alkaloids called haminols, such as haminol-A and haminol-B (**Atlas 79, 80**), have been described from several Mediterranean species of *Haminoea* (Cimino, Passeggio, Sodano, Spinella & Villani, 1991). These polyacetate molecules are biosynthesized in a unique manner, using nicotinic acid as a starter unit to which acetic acid units are sequentially added (Fontana, 2006). That they are biosynthesized by the mollusks has been rigorously proved in *Haminoea orbignyana* (**Photo 5**) by using precursors labeled with stable isotopes, deuterated nicotinic acid (Cutignano, Tramice, De Carlo, Villani, Cimino & Fontana, 2003) and acetate labeled at one or both positions with ^{13}C (Cutignano, Cimino, Giordano, d'Ippolito & Fontana, 2004).

Haminols have been characterized as "alarm pheromones," as have similar compounds in other cephalaspideans. The animals follow one another's mucous trails. Haminols in the mucus elicit an escape reaction. They are exuded when the cephalaspidean is attacked, but it is thought that they do not repel the predator. The haminols appear to be characteristic of each species of *Haminoea*. Specificity depends upon the distribution of the two or three double bonds along the chain of the molecule. Alarm pheromones are also known from the prosobranch mud snail *Nassarius obsoletus* (Atema & Stenzler, 1977).

From the point of view of evolutionary theory it is somewhat problematic why these snails would inform their conspecifics that they are alarmed, whether by a predator or something else. They are not close relatives and they do not engage in cooperative defense of the group. We have considered this matter before. And one wonders why there is so much specificity to the response. Why should it make a difference when spreading the alarm, to spread it only to conspecifics? The possibility is worth considering, that the secretion has some other function, whether a primary function or an auxiliary one. Among these are species recognition and isolating mechanisms.

In *Philinopsis* (see below) the pheromones are indeed used in locating mates. Haminols have only been found in some species of *Haminoea*, all of them from the Mediterranean. But similar compounds occur in *Navanax*. *Haminoea callidegenita* contains a series of alkylphenols (**Atlas 81**) that are similar to navenone-C (**Atlas 67**) (Spinella, Alvarez & Cimino, 1998; Marin, Alvarez, Cimino & Spinella 1999) and are therefore suspected of being alarm pheromones. *Haminoea fusari* contains, in addition to several haminols, fusaripyrones, which have an unusually long, 11-subunit chain of propionate units (**Atlas 154-157**) (Cutigano, Blihoghe, Fontana, Villani, d'Ippolito & Cimino, 2007). The starter unit could be propionate and elongated with additional propionate units, but acetate chain formation followed by subsequent methylation has not been excluded.

In the family Smaragdinellidae, *Smaragdinella calyculata* (**Photo 10**) contains the 2-alkylpyridine naloamine (**Atlas 82**) and the polypropionate nalodionol (**Atlas 118**) (Szabo, Nakao, Yoshida & Scheuer, 1996). This makes it very much like *Bulla*. *Smaragdinella*, which lives high up in the intertidal zone, shows a greater degree of shell reduction than does *Haminoea*. Usually cephalaspideans contain either polyacetates or polypropionates, but some contain both. *Smaragdinella*, like *Haminoea*, is an example of the latter. They are located in different parts of the body and perhaps function differently.

The carnivorous cephalaspideans in which gizzard plates are present or have been secondari-

ly lost are generally placed in the superfamily Philinoidea, which is subdivided into the families Cylichnidae and Aglajidae. The former contain the more primitive forms with well-developed shells, but often the shell is considerably reduced. The latter have undergone more shell reduction, and the gizzard plates are lost.

Let us consider the Cylichnidae first. In *Philine aperta* as well as other species in the same genus, sulphuric acid from the surface of the body deters predation by fish (Thompson, 1960, 1986). In *Scaphander lignarius,* and supposedly in others of its genus, such as *Scaphander japonicus* (**Photo 11),** there are glands within the mantle cavity comparable to those of other cephalaspideans and anaspideans that produce secretions for which defensive functions have been implicated (Perrier & Fischer 1908, 1911). Its lignarenones, such as lignarenone-A and lignarenone-B (**Atlas 71, 72**), which are ω-arylmethylketones, have been considered potential alarm pheromones (Cimino, Spinella & Sodano, 1989b). More recently a series of metabolites, either with an additional carbon or else reduced, have also been detected (**Atlas 73-78**) (Della Sala, Cutignano, Fontana, Spinella, Calabrese, Domenech Coll, d'Ippolito, Della Monica & Cimino, 2007).

Lignarenones, by analogy with haminols, should be biosynthesized by the mollusk according to a polyketide pathway that involves benzoic acid or cinnnammic acid as the starter unit and acetate in the elongation step. Recently it has been rigorously proved that lignarenones are biosynthesized by the mollusk. Using stable isotopes, clear labeling was detected in the aromatic ring and in the alkyl chain (Cutignano, Avila, Domenech-Coll, d'Ippolito, Cimino & Fontana, 2008). In particular, benzoic acid is elongated with two units of acetate, one of propionate, and then one of acetate, which is intact in lignarenone C (**Atlas 73**), but loses a carbon in lignarenones A and B (**Atlas 71, 72**). At least these polyketides are very similar in structure to the alarm pheromones of *Navanax* (such as navenone-B) (**Atlas 66**).

The family Aglajidae has been extensively studied from a chemical perspective, especially the genera *Philinopsis* and *Navanax*. As already mentioned, these animals often feed upon other opisthobranchs, and obtain metabolites such as polypropionates from them. They also contain alarm pheromones. Navenones A, B, and C (**Atlas 65-67**) were first described from *Navanax inermis* (**Photo 13**) (Sleeper & Fenical, 1977). They occur in the bright yellow secretion of a specialized gland, which has a location that is not optimally positioned for repelling predators. *Navanax* locates both prey and mates by following another animal's slime trail. When disturbed it secretes large quantities of the pheromones into the slime trail, and other individuals react by avoidance maneuvers when they contact it. The slugs are cannibalistic, and secretion was not elicited when a small animal was attacked by a larger one (Sleeper, Paul & Fenical, 1980).

In an experimental effort to see if *Navanax inermis* was the source of the navanones in its own body, Fenical, Sleeper, Paul, Stallard and Sun (1979) got some incorporation of sodium acetate labeled with ^{14}C, suggesting that the pheromones are produced by the animals themselves. However, the experiments designed to test for synthesis did not rigorously exclude the possibility of dietary origin of the metabolites. It would seem, therefore, that polypropionates collected in *Philinopsis* and *Navanax* should derive them by feeding on other cephalaspideans, in particular *Bulla*. Propionates that occur in *Bulla gouldiana* occur in *Navanax inermis* (**Atlas 113, 117**) (Spinella, Alvarez & Cimino, 1993). They are assumed to be defensive, but this has not been tested.

The picture is more complete for the Mediterranean *Bulla striata* (**Photo 9**) and the cephalaspideans that feed upon it. Recent evidence has shown that *B. striata* biosynthesizes aglajnes (**Atlas 114-116)**) making it a very plausible source for these metabolites (Fontana, Cutignano, Giordano, Domenèch Coll & Cimino, 2004). Polypropionates derived from *B. striata* occur as defensive metabolites in *Philinopsis depicta* (**Photo 12**) (Cimino, Sodano & Spinella, 1987; Cimino, Sodano,

Spinella & Trivellone, 1985). Naturally the metabolites are quite similar. *Philinopsis speciosa* from Hawaii contains the polypropionate metabolites niuhinone A and B (**Atlas 112, 113**), as well as a pyridine derivative pulo'upone (**Atlas 69**) (Coval, Schulte, Matsumoto, Roll & Scheuer, 1985; Coval & Scheuer, 1985) the source of which is unknown but which may reasonably be assumed to be cephalaspideans. An isomer of pulo'upone (**Atlas 70**) has been found in *Bulla gouldiana* and *Navanax inermis* (Spinella, Alvarez & Cimino, 1993).

In *Philinopsis speciosa* there have also been found a depsipeptide, kulolide-1 (**Atlas 633**) (Reese, Gulavita, Nanda, Nakao, Hamann, Yoshida, Coval & Scheuer, 1996), a linear tetrapeptide, pupukeamide (**Atlas 634**) (Nakao, Yoshida & Scheuer, 1996), additional peptides (**Atlas 635**) and the macrolide tolytoxin-23-acetate (**Atlas 662**) (Nakao, Yoshida, Szabo, Baker & Scheuer, 1998). The structure of these metabolites suggests that they are ultimately derived from cyanobacteria, probably via anaspideans and perhaps other opisthobranchs that graze upon them.

The family Gastropteridae consists of a few species of small animals that, so far as known, feed upon sponges. *Sagaminopteron psychedelicum* and *Sagaminopteron nigropunctatum* have been found to feed upon a sponge that is closely related to *Dysidea granulosa* (Demospongiae: Dictyoceratida: Dysideidae) and to contain defensive metabolites (Becerro, Starmer & Paul, 2006). The sponge contains three polybrominated diphenyl ethers, of which one, 3,5-dibromo-2-(2′,4′-dibromophenoxy)phenol (**Atlas 177**), is concentrated in the parapodia of the slugs. *Sagaminopteron psychedelicum* (**Photo 15**) is strikingly aposematic, as is *Sagaminopteron ornatum*, shown in **Photo 14**. *Sagaminopteron nigropunctatum*, however, is not aposematically colored.

Both Anaspidea and Sacoglossa have "primitive" genera in which the shell is relatively well developed, and in which there is a head-shield and other "typical" cephalaspidean anatomy. They are excellent examples of genera that are transitional between one order and another. This was recognized long ago, and it was only the tradition of gradal classification that kept *Akera* out of Anaspidea and *Cylindrobulla* and others out of Sacoglossa. Both anaspideans and sacoglossans are obviously highly modified herbivorous cephalaspideans, but we must remember that herbivory is a plesiomorphy so it does not tell us anything about their relationships. The gizzard in Anaspidea does not display such specializations as three major plates that are characteristic of the "higher" cephalaspideans, and in Sacoglossa its absence could mean either that it has never been present or that it has been lost because of the liquid diet. The place to look for a link to cephalaspideans and for evidence of a relationship between Anaspidea and Sacoglossa is therefore among such obscure little cephalaspideans as *Diaphana*.

The molecular evidence published to date is not very encouraging with respect to this problem, as is true of many of the "deeper" relationships. This situation is exacerbated because, although there is an excellent sample of anaspidean sequence, including the primitive genus *Akera*, data for sacoglossans are sparse, and the shelled forms, and most importantly the most primitive among these, are sometimes not included. That makes it very likely that there will be "long branch attraction," which tends to cause a lineage to slip out of place and connect to a distant branch. Data on 16S RNA are available for *Diaphana minuta* (Wägele, Vonnemann & Wägele, 2003: 21 figures 8.3 and 8.4). This animal appears at the base of a clade made up of the higher cephalaspideans and is the sister group of Anaspidea in a parsimony analysis. However, in a distance analysis it is considerably more distant, and Anaspidea and Sacoglossa appear as sister groups, the cephalaspideans and sacoglossans in effect switching positions. COI (cytochrome oxidase I) sequence studies show the cephalaspideans closer to the anaspideans, but the sacoglossans not far distant. The position of Sacoglossa is much more distant in other molecular trees, but these are not consistent with one another in their position (Dayrat, Tillier, Lecointre & Tillier 2001; Thollesson, 1999). The molecular evidence therefore does not provide much support for an anaspidean-sacoglossan relationship,

but it does not provide grounds for rejecting one either.

As to more traditional evidence processed in a cladistic spirit, it is interesting that Mikkelsen (2002) places the anaspideans and higher cephalaspideans together with the nudibranchs and notaspideans, with the sacoglossans as the sister group. Gizzard plates are scored as lacking in sacoglossans, even though that condition may be secondary. The shell adductor muscles that are found in the sacoglossan *Cylindrobulla* and in the anaspidean *Akera* are considered to have evolved twice, and they are not scored in the shell-less relatives of those two genera in which they have been, or may have been, lost. Such character conflicts are the inevitable result of the methodology. Of course one can argue that letting the majority of characters determine the results compensates for the small number of characters used and the inability to deal with parallel evolution and secondary loss.

Actually the shell adductors are part of an entire suite of characters that, together with others, have been used in the past to connect Anaspidea and Sacoglossa with each other and with their possible relatives within the Cephalaspidea. The connection between Anaspidea and *Diaphana* and its allies was made on the basis of the shell structure by Thiele (1935) and on the basis of soft-part anatomy by Odhner (1939). More recently a close relationship between Sacoglossa and the Diaphanidae has been maintained by Jensen (1996a). A proper understanding of the shell and its associated musculature depends upon knowing how the whole functional system works. Among opisthobranchs in general, the reduction of the shell has been accompanied by an increase in the amount of protein and carbohydrate, making it more flexible and less fragile (Poulicek, Voss-Foucart & Jeuniaux, 1991). In *Akera* and the shelled sacoglossan *Oxynoe*, the protein is rich in hydroxyproline, an amino acid that is characteristic of collagen, evidently lending the protein and the shell an extra degree of flexibility (Ghiselin, Degens, Spencer & Parker, 1967). The ability of the shell to bend is facilitated by an uncalcified, membranous area adjacent to where the rim of the shell connects to the main whorls. Contraction of adductor muscles narrows the opening, and in some cases has been observed to produce a respiratory current. In anaspideans, such an arrangement occurs only in *Akera*, and understandably, since the shell has been much further reduced in all the others. In sacoglossans it occurs in *Cylindrobulla*, which is thought to be the sister group of the rest of them, implying again that it was present in the ancestors of the ones without shells or in which the shells have been much reduced.

The similarity in structure of the shells in *Akera* and *Cylindrobulla* is quite striking when they are viewed from the apex. However, such a condition is maintained in many shelled sacoglossans, such as *Ascobulla* and, as already mentioned, *Oxynoe*. In one lineage of shelled sacoglossans, the so-called "bivalved gastropods," the shell has been subdivided, giving an animal that looks very much like a clam. The adductors now close the valves by pulling them together. The exact homologies of the muscles have not been worked out, but that is irrelevant to the functional and structural continuity of the arrangement in Anaspidea and Sacoglossa. The only remaining question has to do with the relationship of *Diaphana* and its relatives to these groups. In fact, the shell of *Diaphana* looks very much like that of *Akera*, and it is possible that it occupies a position on the branch that led to the anaspideans. On the other hand, the best molecular tree that we have for cephalaspideans places *Diaphana* at the base of Cephalaspidea *s.s*. (Malaquias, Mackenzie-Dodds, Gosliner, Bouchet & Reid 2009), and not closely related to either Anaspidea or Cephalaspidea. It is possible that the aforementioned anatomical similarities are plesiomorphies, though some might want to treat them as convergent. For our discussion of the evolution of chemical defense, all that matters is that Sacoglossa and Anaspidea are only distantly related to Cephalaspidea in the strict sense and that the common ancestor was probably herbivorous.

Chapter VII

Anaspidea and Pteropods

The Anaspidea, or sea-hares, form a distinct group, the naturalness of which has never been contested. They can easily be recognized by the presence of two pairs of rolled rhinophores, which are sensory structures derived by modification of the head-shield. Sacoglossans also have rolled rhinophores, but only a single pair. The systematics of the order is fairly straightforward and roughly approximates the evolutionary relationships. Molecular studies tend to substantiate the system that was worked out by traditional systematists (Martinez, 1995; Medina & Walsh, 2000, 2001; Medina, Collins & Walsh, 2005; Klussmann-Kolb, 2004). Although the precise relationships are not always well resolved, we will present tree diagrams in the text that close enough approximations for our purposes, especially since the natural products of many of the species have not been studied. *Akera*, as discussed in the last chapter, forms a nice morphological transition to Cephalaspidea. As can be seen from **Photo 16**, which depicts the animal swimming by means of parapodial lobes, the cephalic shield is like that of cephalaspideans and there are no rhinophores. *Akera* feeds upon green algae, but no data on secondary metabolites have been reported in the literature. It does produce a purple secretion (Thompson & Seward, 1989). This genus forms the sister group of all the rest, which consist of two major lineages, variously classified.

Here we will follow an old-fashioned arrangement and treat each of these three groups as families (Akeridae, Aplysiidae, and Dolabellidae), the first constituting a superfamily Akeroidea and the second two the superfamily Aplysioidea. The family Aplysiidae contains a single subfamily, Aplysiinae, consisting of the genus *Aplysia* and the rather aberrant *Syphonota*. In the Aplysiinae the body is relatively high and the parapodia, which are extensions of the foot, provide partial coverage for a mantle cavity containing gills and a rudimentary shell. The second lineage is the third family, Dolabellidae, subdivided into three subfamilies.

Akeroidea
- Akeridae
  - *Akera*

Aplysioidea
- Aplysiidae
  - Aplysiinae
    - *Syphonota*
    - *Aplysia*
  - Dolabellidae
    - Dolabellinae
      - *Dolabella*
    - Notarchinae
      - *Notarchus*
      - *Bursatella*
      - *Stylocheilus*
    - Dolabriferinae
      - *Dolabrifera*
      - *Petalifera*
      - *Phyllaplysia*

We will follow this sequence in the text, in which trees are also presented.

Anaspideans are all herbivorous. With few exceptions they feed upon macroscopic algae, at least as adults. Because they feed upon a wide range of algae, anaspideans have been a rich source of metabolites of interest to natural products chemists. A great deal is known about their feeding biology. Food is ingested by means of a radula aided by jaws, then passed to a crop and gizzard where it is triturated by heavy, irregular plates that are strengthened by carbohydrates but not calcified. Subsequently the food is moved to the digestive glands where it is absorbed. Secondary metabolites from the food are dealt with in the digestive gland, where they and their breakdown products may occur in high concentrations. Older work on the biology of anaspideans, including their feeding and metabolites, was admirably reviewed by Carefoot (1987). However, many of the

metabolites have little relevance to the basic theme of this monograph, which is chemical defense. Therefore we focus mainly on the more recent literature, and do not provide as detailed coverage as we have for other groups.

The toxicity of anaspideans was known in classical antiquity (Caprotti, 1977). According to Pliny the Elder, the Romans used them for inducing abortions. The *Lepus Marinus* is listed among poisonous animals by the seventeenth-century Jesuit polymath Athanasius Kircher (1665, vol. 1, p. 144). Sakamoto, Nakajima, Misawa, Ishikawa, Itoh, Nakashima, Okanoue, Kashima and Tsuji (1998) discussed a case of acute liver damage in a patient who had ingested *Aplysia kurodai*. This case is unusual, but by no means unique (Sorokin, 1988). Sea-hare flesh is not commonly eaten by human beings, and that it is not palatable should be obvious from its smell. Mazzarelli (1893:48) cites Rang for *Dolabella* being eaten by humans at Tubai, in the Society Islands. According to a personal communication from Michael Hadfield and Marilyn Switzer-Dunlap to Pennings and Paul (1993), the natives of some Pacific islands do eat them, but only after removing the viscera. Kamiya, Sakai and Jimbo (2006:216) remark that in Japan "people on Okinoshima Island eat cooked local species of *Aplysia* as a delicacy after removing the viscera and washing the body surface with common salt." These authors also review poisoning due to the ingestion of sea-hare eggs, which have been used in traditional medicine.

Various non-human animals can and do eat sea-hares, but they avoid the parts in which secondary metabolites are concentrated. Winkler and Tilton (1962) discuss the sea anemone *Anthopleura xanthogrammica* preying on *Aplysia californica*. Mazzarelli (1893) reported feeding by *Dromia* and other brachyuran crabs on *Aplysia* and their eggs, but said that these were the only predators of *Aplysia* known to him. Pennings (1990a) fed *Aplysia californica* on *Ulva* (without metabolites) and *Plocamium* (with metabolites) to fish. He found that those fed with *Plocamium* had more of an emetic effect than those fed on *Ulva*, yet the fish still rejected those fed on *Ulva*. Pennings, Nastich, and Paul (2001) tried raising *Stylocheilus* on diets with and without metabolites and then feeding them to fish: there was little evidence of deterrence, and which fish ate the slugs seemed to depend upon the species of fish. On the other hand there are experiments conducted by Eisner (reported in Kinnel, Dieter, Meinwald, Van Engen, Clardy, Eisner, Stallard & Fenical, 1979) on *Aplysia brasiliana*. Sharks, which ignored the slugs that were offered as food, were tricked by sandwiching chunks of slug between fish fillets. The sharks spat out the slug meat. However, they did eat the buccal mass, an internal organ in which one might well expect defensive metabolites to be absent.

In addition to metabolites in the digestive gland, reproductive system and skin of anaspideans, there are also some secretions from glands located in the mantle cavity near the gill that have long been implicated in defense. These secretions include the purple material that the animal often exudes when disturbed, as is commonly observed by even casual visitors to the shore. The evolutionary history of the glands that produce these secretions has been extensively discussed in the scientific literature. In prosobranchs there is an organ called the "hypobranchial gland," which helps to keep the gill free of sediment. It is also implicated in producing the halogenated alkaloids that occur in some prosobranchs (Baker, 1974; Benkendorff, Bremer & Davis, 2000). According to an excellent review by Hoffmann (1932-1940), a hypobranchial gland is present in some cephalaspideans, including *Acteon* and *Scaphander*. It also is said to occur in anaspideans and sacoglossans that retain a well developed shell, and likewise in Thecosomata (Wägele, Ballesteros & Avila, 2006). The mantle cavity opened out when the shell became reduced, and water could now flow over the gill and cleanse it without the need of the secretions of the hypobranchial gland. New glands then arose from the epithelium of the mantle, becoming larger and more localized, and undergoing a division of labor. There are two kinds of secretion, one opaline or whitish, the other

usually purple. The former is produced by the opaline gland (gland of Bohadsch), which is generally considered unique to the Anaspidea (Nolen & Johnson, 2001). Ghiselin (1963), following earlier authors, referred to a problematic organ in the cephalaspidean *Runcina* (=*Metaruncina*) *setoensis* as an opaline gland, as did Baba (1967). This homology is dubious. The gland produces a whitish secretion, but it does not appear to be used defensively. The glands of Blochmann in cephalaspideans do not produce ink, but they do produce certain other secretions, and are sometimes considered homologous to the ink glands of anaspideans. The opaline glands are perhaps a locally differentiated derivative of Blochmann's glands.

Both secretions are thought to be defensive, but they function in somewhat different ways and have different chemical properties. The opaline secretion provides for defense from crabs (Mazzarelli (1893). Kamiya, Muramoto, Goto, Sasakai, Endo and Yamazaki (1989) extracted a thermolabile protein from the opaline gland secretion of *Aplysia juliana*. It was toxic to crabs but showed no antibacterial activity. The purple secretion is likewise defensive. Although this secretion is not purple in *Aplysia juliana*, Kamiya, Muramoto, Goto, Sakai, Endo and Yamazaki (1989) found that it was likewise toxic to crabs and had strong antibacterial activity as well. Yamazaki, Tansho, Kisugi, Muramoto and Kamiya (1989) isolated a cytolytic protein from the purple secretion of *Dolabella auricularia*. Kicklighter, Shabani, Johnson and Derby (2005) studied the interactions between *Aplysia californica* and a spiny lobster that preys upon it, *Panulirus interruptus*. These authors point out that chemical defense mechanisms may function in ways that would not be revealed by experiments that merely test the reactions of predators to the chemicals. Of course if the assay organism is a fish rather than a crustacean, negative results might be misleading. The secretions have various effects on the lobster, stimulating appetitive and feeding behavior and also evoking escape responses and grooming. The opaline gland also contains a chemical deterrent that suppresses feeding. The lobster is deceived into attending to a false food stimulus, and the sensory mechanisms involved in feeding are disrupted. The inking response has been compared to that of cephalopods, which, however, is directed against visual predators. Instead of looking like food, the anaspidean secretions taste and smell like food (Derby, 2007). L-amino acid oxidases such as escapin are enzymes that oxidatively deaminate l-amino acids, rapidly releasing a variety of active compounds, including hydrogen peroxide, ammonium ions, α-keto acids, and carboxylic acids. L-lysine is oxidized by escapin to various products (Kamiya, Sakai & Jimbo, 2006). The enzyme is present in the ink gland, whereas its substrate is concentrated in the opaline gland. The reactions occur when the two are released together and mixed, giving products that suppress feeding by crustaceans (Derby, 2007).

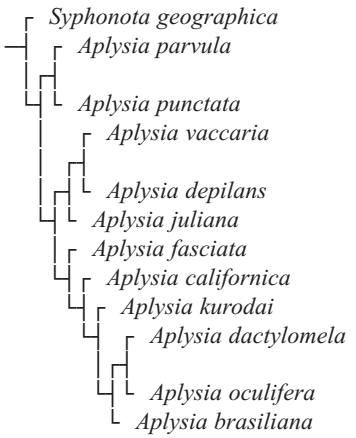
Since anaspideans of the genus *Aplysia* are abundant and readily obtained, and since they are also familiar as classical materials for neurophysiological and behavioral research (Kandel, 1979), it is hardly surprising that much of the early work on defensive metabolites was done on them. On top of that, a wide variety of metabolites turned up, giving rise to an extensive literature. These metabolites are generally derived from food, but their names are often based on the opisthobranchs in which they were found. The food items are almost always algae or cyanobacteria, and the algae include representatives of the three major groups (Chlorophyta, Rhodophyta and Phaeophyta). Green algae, which may be the ancestral food in Anaspidea, are often eaten, but they seem not to be an important source of metabolites in this group. At least they are often said to be the diet of sea-hares that lack dietary defensive metabolites, such as *Aplysia juliana* according to Pennings and Carefoot (1995). Brown algae are commonly eaten as well. But it is from the red algae that much of the dietary secondary metabolites are derived. Often they come from the genus *Laurencia*, for which Eriksen (1983) has written an excellent, if now somewhat dated, review. *Plocamium* is also an important source.

Aplysia, like some other anaspideans, owes much of its ecological success to its ability to feed upon a wide variety of toxic seaweeds, and to deal with the secondary metabolites therein contained. It is particularly adept at dealing with halogenated terpenoids. The wide variety of seaweeds that it consumes is reflected in the diversity of metabolites that can be recovered from animals of a single species. Natural products chemists have created a long list of these, partly by sacrificing a large number of animals, with somewhat unfortunate consequences for conservation (Wessels, König & Wright, 2000). *Aplysia* is considered a generalist herbivore, though of course there are the usual differences among the species in what they eat. However, there is a noteworthy shift from specialization to generalization as the animals get older (Pennings, 1990a, 1990b). In the Mediterranean, as shown by their metabolites, *Aplysia fasciata* feeds upon red algae whereas *Aplysia depilans* feeds upon brown algae.

Most of the metabolites from *Aplysia* have been obtained from the digestive gland, which is where detoxification occurs, and the same is true of other anaspidean genera. As mentioned earlier, this is not the optimal location for them from the point of view of chemical defense. That point has been largely responsible for some biologists downplaying the importance of chemical defense (Pennings, 1994). However, some metabolites do make their way from the gut to places where they are apt to be much more effective. Miyamoto, Higuchi, Marubayashi and Komori (1988) obtained two polyhalogenated monoterpenes in the body wall of *Aplysia kurodai*, together with some unusual degraded sterols (**Atlas 561, 562**). Surprisingly, closely related steroids were also found in exposed portions of the body in both Mediterranean (**Atlas 563, 564**) (Spinella, Gavagnin, Crispino, Cimino, Martinez, Ortea & Sodano, 1992) and Atlantic (**Atlas 565**) (Ortega, Zubia & Salvà, 1997) specimens of *Aplysia fasciata*.

Recently Gavagnin, Carbone, Nappo, Mollo, Roussis, and Cimino (2005) isolated two closely related degraded sterols (**Atlas 566, 567**) from the skin of *Syphonota geographica* (also known as *Aplysia geographica*) (**Photo 23**). Likewise aplyolides A-E (**Atlas 44-48**), which are lactonized dihydroxy fatty acids, were detected in the integument of *Aplysia depilans* (Spinella, Zubia, Martînez, Ortea & Cimino, 1997). There is some evidence that these metabolites, which are known to be toxic to fish, are synthesized *de novo*. Notably, *Siphonota geographica* feeds upon a marine grass, whereas *Aplysia depilans* eats algae. There is ample evidence that the skin of anaspideans is distasteful (De Nys, Steinberg, Rogers, Charlton & Duncan, 1996) and that metabolites are sequestered (Rogers, Steinberg & De Nys, 1995; Rogers, De Nys, Charlton & Steinberg, 2000). The egg masses of *Aplysia juliana* contain unsaturated fatty acids in high enough concentrations to prevent the growth of bacteria (Benkendorff, Davis, Rogers & Bremner, 2005).

The secondary metabolites from *Aplysia* and *Syphonota* are known from thirteen of the approximately forty species within the family Aplysiidae. Even so, only a few of these species have been thoroughly studied. Furthermore, the metabolites are largely derived from the plants upon which the animals feed, and only a few of these would seem to play an important role in defense. Nonetheless an effort to organize the material phylogenetically seems desirable. We will therefore survey the various species using the following tree, which is a fair approximation to the classification of Eales (1960) and to the findings of recent research:



Syphonota is the sister group of *Aplysia*. We have already mentioned the degraded sterols of *S. geographica* (Gavagnin, Carbone, Nappo, Mollo, Roussis & Cimino, 2005; Carbone, 2007; Carbone, Gavagnin, Mollo, Bidello, Roussis & Cimino, 2008). Noteworthy are two new degraded sterols, aplykurodinones 1 and 2 (**Atlas 566, 567)**. They occur in the mantle, not the gut. Such unusual compounds are known from the mantles of other anaspideans, namely *Aplysia fasciata* and *A. kurodai* (see below). These species are not closely related, and they occur in widely separated geographical areas. Although feeding upon algae is the usual pattern among anaspideans, *S. geographica* has been found to contain large amounts of a sea-grass, *Halophila stipulacaea*, in its stomach. Quite a number of metabolites apparently derived from this vascular plant have been recovered from the digestive gland. The main secondary metabolite of both the sea-grass and the slug is syphonoside (**Atlas 450**), a macrocyclic glycoterpenoid (Gavagnin, Carbone, Amodeo, Mollo, Vitale, Roussis & Cimino, 2007).

The first branch of the tree contains two species, *Aplysia parvula* (shown swimming in **Photo 18**), and *A. punctata*, which form a well-recognized clade. *Aplysia parvula*, studied in Guam, was found to feed preferentially upon the red alga *Portieria hornemannii*, which contains variable amounts of the halogenated monoterpenes apakaochtodene A and B (**Atlas 190-191**). The slugs, which were somewhat deterred from feeding when metabolite concentration was high, nonetheless accumulated them and were shown experimentally to be defended from fish predation (Ginsburg & Paul, 2001). *A. parvula* has been found to contain, in its digestive gland, aplyparvunin (**Atlas 38**), a brominated acetogenin that is a dicyclic ether (Miyamoto, Ebisawa & Higuchi, 1995). It also contains a halogenated cyclic acetogenin, (3*Z*)-bromofucin (**Atlas 28**) (MacPhail & Davies-Coleman, 2005). Specimens studied in New Zealand were found feeding on the red alga *Plocamium costatum* (, Appleton & Copp, 2005). The main metabolite in the alga, a brominated and chlorinated terpenoid called costatone (**Atlas 202**), was found to be fourteen times as concentrated in the slug, almost all of it in the digestive gland. Although the metabolite evidently is not optimally positioned for defense, large fish that ingested the slugs rapidly rejected them.

The closely related *Aplysia punctata* is likewise noteworthy for halogenated monoterpenes. A series of seven of these were described by Quiñoa, Castedo and Riguera (1989) and several more (**Atlas 194-199**) by Ortega, Zubía, and Salvà (1997). They were recovered from the animals' defensive secretion and seem to be derived from the red alga *Plocamium coccineum*. There are also epidioxy sterols (Jiménez, Quiñoa, Castadeo & Riguera, 1986).

Aplysia juliana, *A. vaccaria*, and *A. depilans* have been placed in the same subgenus together with some species the secondary metabolites of which are as yet unknown.

Aplysia juliana was found under certain circumstances to feed only on the green alga *Ulva* and to contain no metabolites (Rogers, Steinberg & De Nys, 1995). Kobayashi, Kanda and Kamiya (1991) briefly noted two pyropheophorbides from the viscera of this species. A halogenated diterpenoid lactone, angasiol acetate (**Atlas 372**), has also been recorded from this species (Atta-ur-Rahman, Alvi, Abbas, Sultana, Choudhary & Clardy, 1991).

Aplysia vaccaria was found to contain non-halogenated diterpenoids called crenulides in its digestive gland (Midland, Wing & Sims, 1983), typical of the brown alga *Dictyota crenulata*. One of these, acetoxycrenulide (**Atlas 371**), is highly toxic to fish (Sun, McEnroe & Fenical, 1983).

Aplysia depilans has a larger number of known metabolites than do the above species, perhaps because it has been more intensively studied. On the other hand it has not been a particularly rich source of them, and this may have something to do with the animals' diet. We have detected metabolites from brown algae in this species. Quiñoa, Castedo, and Riguera (1989) found no halogenated compounds in this species. They found only green algae in the specimens of *A. depilans* that they studied. *Aplysia depilans* does, however, contain non-halogenated diterpenes, such as dictyol-A (**Atlas 370**), one of a series of perhydroazulene diterpenes so named because they occur in brown algae of the family Dictyotaceae (Minale & Riccio, 1976). *A depilans* has also been a source of steroids (Lupo di Prisco & Dessi Fulgheri, 1973; Voogt & Van Rheenen, 1973). Jiménez, Quiñoa, Castedo and Riguera (1986) record peroxy sterols from this species as well as from *A. punctata* (noted above).

Aplysia fasciata is noteworthy for having a variety of ichthyotoxic degraded sterols. These include 4-acetylaplykurodin-B (**Atlas 563**) and aplykurodinone B (**Atlas 654**) (Spinella, Gavagnin, Crispino, Cimino, Martinez, Ortea & Sodano, 1992), the defensive importance of which has already been discussed above, and a C-3 epimer of the latter compound, 3-*epi*-aplykurodinone B (**Atlas 565**) (Ortega, Zubía & Salvà, 1997). Halogenated monoterpenes have also been found in this species (identified as *Aplysia limacina*) (Imperato, Minale & Riccio, 1977). In the Mediterranean these animals have been found to feed upon red algae.

Aplysia californica undergoes metamorphosis on various red algae, including *Rhodomenia californica*, *Agardhiella subulata*, *Laurencia pacifica*, *Corallinia officinalis*, *Plocamium cartilagineum*, and *Gracilliaria* sp. (Pawlik, 1989; Nadeau, Paige, Starczak, Capo, Lafler & Bidwell, 1999). Although red algae are more effective in inducing metamorphosis, brown algae (*Dictyopteris undulata*, *Pachydiction coriaceum*) and even green algae (*Ulva*) have been shown to work too. The animals have been found to contain halogenated monoterpenes derived from red algae of the genus *Plocamium,* upon which these animals feed. The first of these to be isolated was (3*R*,4*S*,7*S*)-*trans*,*trans*-3,7-dimethyl-1,8,8-tribromo-3,4,7-trichloro-1,5-octadiene (**Atlas 188**) (Faulkner, Stallard, Fayos & Clardy, 1973). Another was 7-chloro-3,7-dimethyl-1,4,6-tribromo-1-octen-3-ol (**Atlas 189**) (Faulkner & Stallard, 1973). Subsequent investigations turned up quite a number of halogenated terpenoids from the digestive glands of *A. californica*, some of which could not be found in the local algae which were sampled as plausible sources (Stallard & Faulkner, 1974; Ireland, Stallard, Faulkner, Finer & Clardy, 1976). Some of the compounds were found to have been transformed within the digestive glands (Stallard & Faulkner, 1974).

Aplysia kurodai has received considerable attention from Japanese investigators, no doubt in part because of its availability. It was found to contain three cytotoxic polyketide macrolides, aplyronine A, aplyronine B and aplyronine C (**Atlas 663-665**) (Yamada, Ojika, Ishigaki & Yoshida, 1993). Halogenated monoterpenes have been recovered from the digestive gland (Ksumi, Uchida, Inouye, Ishitsuka, Yamamoto & Kakisawa, 1987). Two halogenated monoterpenes have been isolated from parts of the body where they are likely to function in chemical defense (Miyamoto, Higuchi, Marubayashi & Komori, 1988; Inouye, Uchida, Kusumi & Kakisawa, 1987). These are a

cyclic molecule, aplysiaterpenoid-A (**Atlas 192**) and a linear one, aplysiaterpenoid B (**Atlas 193**). Another halogenated monoterpene from *A. kurodai* is kurodainol (Katayama, Ina, Nozki & Nakamura, 1982). The halogenated diterpenes, aplysin-20 (**Atlas 364**) (Yamamura & Hirata, 1963, 1971) and isoaplysin-20 (Yamamura & Terada, 1977) have also been recorded from this species. Another is the brominated diterpenoid aplysiadiol (**Atlas 365**) (Okija, Yoshida, Okumura & Ieda 1991). There are also some degraded sterols, aplykurodin A and B (**Atlas 561, 562**) (Miyamoto, Higuchi, Komori, Fujioka & Mihashi, 1996). *A. kurodai* has also been the source of several new alkaloids. The phenolic alkaloids aplaminone, neoaplaminone, and neoaplaminone sulphate (**Atlas 166-168**) are said to be cytotoxic but their locality in the animal was not specified by the authors (Kigoshi, Imamura, Yoshikawa & Yamada, 1990). Aplydilactone (**Atlas 43**) is a dieicosanoid lactone (Okija, Yoshida, Nakayama & Yamada, 1990). Aplysepin (**Atlas 620**), a 1,4-benzodiasepine alkaloid, was recovered from whole body extracts (Ojika, Yoshida & Yamada, 1993).

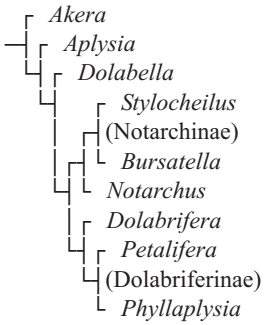
Aplysia brasiliana, the repugnancy of which to sharks has been discussed above, contains the acetylenic chloroether brasilenyne (**Atlas 26)** and another haloether, *cis*-dihydrophytin (**Atlas 27**) (Kinnel, Dieter, Meinwald, Ven Engen, Clardy, Eisner, Stallard & Fenical, 1979). Also distasteful to sharks is panacene, an aromatic bromoallaene (Kinnel, Duggan, Eisner, & Meinwald, 1977). *A. brasiliana* is the source of two bromosesquiterpenes, brasudol (**Atlas 280**) and isobrasudol (**Atlas 281**) (Dieter, Kinnel, Meinwald & Eisner, 1979). Non-halogenated, rearranged sesquiterpene alcohols were also isolated from it (Stallard, Fenical & Kittredge, 1978). They probably were derived from the red alga *Laurencia*. Aurisides (**Atlas 55, 56**), which are macrocyclic fatty acid lactones, also occur in it (Sone, Kogoshi & Tamada, 1996).

Aplysia oculifera has been found to contain srilankenyne (**Atlas 35**), an acetylenic C-15-tetrasubstituted tetrahydropyran, surmised to be derived from the red alga *Laurencia* (De Silva, Schwartz, Scheuer & Shoolery, 1983). Two brominated, isomeric acetylenes (**Atlas 36, 37**) have also been recorded from the digestive gland (Schulte, Chung & Scheuer, 1981).

Aplysia dactylomela has been the most prolific source of secondary metabolites in its genus. One indication of its being chemically defended is that it is mimicked by a fish (Heck & Weinstein, 1978). With respect to polyketides evidently derived from red algae, *A. dactylomela* has proven a particularly rich source of cyclic, often acetylenic, ethers. The source is indicated in the name of the dibromochloro ether dactylyne (**Atlas 25**) (McDonald, Campbell, Vanderah, Schmitz, Washecheck, Burks & Van Der Helm, 1975). An isomer of this compound, isodactylyne, has also been recorded (Vanderah & Schmitz, 1976). A group of these that resemble some algal ethers were recovered from the digestive gland (Gopichand, Schmitz, Shelly, Rahman & Van Der Helm, 1981). A series of such ethers (**Atlas 29-34**) have been described by Manzo, Ciavatta, Gavagnin, Puliti, Mollo, Guo, Mattia, Mazzarella and Cimino (2005). Three of these proved to be new: (-)-3*E*,6*R*,7*R*-pinnatifidenyne, (+)-3*E*,6*R*,7*R*-obtusenyne, and (+)-3*Z*,6*R,7R*-obtusenyne. These were enantiomers of known compounds. Two previously known ones were also found: (+)-3*E*-pinnatifidenyne (**Atlas 33**) and (+)-laurenyne (**Atlas 34**). The former had been found in the red alga *Laurencia pinnatifida*, the latter in *L. obtusa*. In addition, these authors found (+)-brasilenol (**Atlas 225**), which is a sesquiterpene ether known from *L. obtusa* and *Aplysia brasiliana*. It was later found that Aplysiols A (**Atlas 63**) and B (**Atlas 64)** are the main triterpene polyethers in the mantle (Manzo, Gavagnin, Bifulco, Cimino, Di Micco, Ciavatta, Guo & Cimino, 2007). These are closely similar, but not identical, to metabolites of *Laurencia*. Other sesquiterpene ethers that have been found in *Aplysia dactylomela* are dactyloxene-B (**Atlas 224**) (Schmitz & McDonald, 1974; Schmitz, McDonald & Vanderah, 1978) and dihydroxydeodactol monoacetate (Schmitz, Michaud & Hollenbeak, 1980). Dactylallene (**Atlas 39**) is a halogenated bicyclic C-15 ether (Ciavatta, Gavagnin, Puliti, Cimino, Martínez, Ortea & Mattia, 1997). *Aplysia dactylomela*, as its probable

synonym *A. angasi*, was the source for aplysiastatin, a brominated polyether sesquiterpenoid that is evidently derived from an alga of the genus *Laurencia* (Pettit, Herald, Allen, Dreele, Vanell, Kao & Blake, 1977). Other halogenated sesquiterpenes have also been recorded (Ichiba & Higa, 1986; Kaiser, Pitombo & Pinto, 1998; McPhail, Davies-Coleman, Copeley & Eggleston, 1999). There is also a series of brominated diterpenes, such as parguerol (**Atlas 367**), deoxyparguerol, and isoparguerol (Schmitz, Michaud & Schmidt, 1982). *Aplysia dactylomela* has been found to contain two molecules consisting of a diterpenoid part linked to an aromatic ring, stypoldione and (+)-epitaondiol (**Atlas 171**) (Gerwick, Fenical, Van Eagen & Clardy, 1979). These compounds, present in the alga *Stylopodium* upon which the juvenile sea-hares were observed feeding, are concentrated in the animals. Another compound, 3-ketoepitaondiol (**Atlas 172**) was present only in the sea-hares, providing evidence for biotransformation. Isolated from the gut of *A. dactylomela*, and from the alga *Cladophora fascicularis* upon which it was observed feeding, was the biologically active diphenyl ether 2-(2′,4′dibromophenoxy)-dibromoanisole (**Atlas 173**) (Kiniyoshi, Yamada & Higa, 1995).

The remaining Anaspidea have been classified in the outline above as the family Dolabellidae, with three subfamilies: Dolabellinae, Notarchinae and Dolabeliferinae. Dolabellids tend to be rather flattened animals, and the parapodia are relatively smaller than they are in *Aplysia*. The following tree better expresses their likely phylogenetic relationships:



The genus *Dolabella* itself makes up the first branch. There may be more than two species, but the only ones that have been studied from the point of view of ecology and natural products chemistry are the Indo-Pacific *Dolabella auricularia* and the Eastern Pacific *D. californica*, which may be a synonym. **Photo 19** shows a specimen of *D. auricularia* resting on sand. When encountered among rocks and on algae they are inconspicuous. The head with its four rhinophores is on the right. Toward the left is an opening through which the respiratory current exits. They are tropical to subtropical animals, and they feed much like *Aplysia* on a wide variety of algae. Pennings and Paul (1992) treat them as generalized, nocturnal grazers. The metabolites are mainly the sort that one might expect to recover from an algivorous opisthobranch, but some of them, including polypropionates and proteins, are clearly of endogenous origin (see also Pennings, Paul, Dumbar, Hamann, Lumbang, Novack & Jacobs, 1999).

In the following survey of the metabolites of *Dolabella* we will not attempt to separate the two putative species. Yamada and Kigoshi (1997) have provided an excellent review of the chemicals. Among polyketides, the cytotoxic macrolides known from *D. aurantica* are noteworthy. Aurisides A and B (**Atlas 55, 56**) are both halogenated (Sone, Kigoshi & Yamada, 1996), as are the polyethers anrilol and enshuol (**Atlas 57, 58**) (Suenaga, Shibata, Takada, Kigoshi & Yomada, 1998). Dolabelides A and B (**Atlas 51, 52**) (Ojika, Naagoya & Yamada, 1995) and C and D (**Atlas 53, 54**) (Suenaga, Nagoya, Shibata, Kishogi & Yamada (1997) are not. Doliculols A and B (**Atlas 41, 42**)

are non-halogenated, acetylenic cyclic ethers (Ojika, Nemoto & Yamada, 1993). Similar cyclic ethers, but halogenated ones, are typical of the red algal genus *Laurencia* and also occur in other sea-hares.

Dolabella is one of the many scattered opisthobranchs in which polypropionates have been detected. *D. auricularia* was the source for very small quantities of the polypropionates known as auripyrone-A and auripyrone-B (**Atlas 120, 121**) (Suenaga, Kigoshi & Yamada, 1996). Supposedly these polypropionates are biosynthesized *de novo* by the slug, for there is no dietary source for them in an herbivorous animal. They occur only in the internal organs. They were detected in very low levels: 1.0 and 1.7 mg. were recovered from 452 kg. of material. A defensive role for them seems most unlikely, but it is anything but obvious what their function might be. They resemble propionates isolated from *Dolabrifera* (see below).

Turning to terpenoid compounds, diterpenes have been found in *Dolabella californica* (Ireland, Faulkner, Finer & Clardy, 1976; Ireland & Faulkner, 1977, 1977). These were the first reports of this skeleton. They are called dolabellanes, and they include dolabelladiene, which is similar to 10-acetoxy-18-hydroxy-2,7,-dolabelladiene (**Atlas 373**). Similar metabolites occur in brown algae of the family Dictyotaceae, indicating the dietary source. A similar molecule, dolatriol (**Atlas 374**), occurs in *Dolabella auricularia* (Pettit, Ode, Harald, Dreele & Michel, 1976). It was also found in what has been called *Dolabella ecaudata* from the Indian Ocean, and therefore probably the same species, together with an apparent monoterpenoid compound, (-)-loliolide (**Atlas 226**) (Pettit, Herald, Ode, Brown, Gust & Michel, 1980). It may be a degraded carotenoid derived from food. The brominated triterpenoid aurilol, a polyether isolated from *D. auricularia* (**Atlas 57**), is similar to compounds known from the red alga *Laurencia* (Suenaga, Shibata, Takada, Kigoshi & Yamada, 1998). Aurisides (**Atlas 55, 56**), which are macrocyclic fatty acid lactones, also occur in it (Sone, Kigoshit & Yamada, 1996).

Dolabella is noteworthy for various linear peptides and cyclic peptides, especially those known as dolastatins, for which there is a large literature (reviewed by Pettit, 1997). They were found to have anti-tumor activity, and one of them, dolastatin 10 (**Atlas 639**), has been found effective in the treatment of human prostate cancer (Turner, Jackson, Pettit, Wells & Kraft, 1988). On the basis of structural analogy it seems likely that these peptides are produced by cyanobacteria that are taken up with the algae on which the slugs feed. The cyclic peptide dolastatin 3 was isolated from *D. auricularia* (Pettit, Kamano, Brown, Gust, Inoue & Herald, 1982). A series of dolastatins, dolastatins 10 (**Atlas 639**) to 15 (**Atlas 640**), have also been described from *D. auricularia* (Pettit, Kamano, Herald, Fujii, Kizu, Boyd, Boettner, Doubek, Schmidt, Chapuis & Michel, 1993). Among these, dolastatin 13 is a biologically active depsipeptide from *D. auricularia* (Pettit, Kamano, Herald, Dufresne, Cerny, Herald, Schmidt & Kizu, 1989). Dolastatin 19 (**Atlas 61**), characterized as a new 14-membered macrocyclic lactone linked to a 2-4,di-*O*-methyl-*L*-α-rhamnoyroside, together with the known macrolides debromoaplysiatoxin and anhydrodebromoaplysiatoxin (see below), was isolated from 600 kilograms wet weight of animals from the Gulf of California (Pettit, Xu, Doubek, Chapuis & Schmidt, 2004). Dolastatin H and isodolastatin H are cytotoxic peptides from *D. auricularia* (**Atlas 644, 645**) (Sone, Shibata, Fujita, Ojika & Yamada, 1996). Dolastatin D is a cytotoxic cyclic depsipeptide from *D. auricularia* (Sone, Nemoto, Ishiwata, Ojika & Yamada, 1993). Dolastatin G and nordolastin G are cyclodepsipetides from *D. auricularia* (Mutou, Kondo, Ojika & Yamada, 1996). Another cytotoxic cyclodepsipeptide from *D. auricularia* is aurilide (**Atlas 641**) (Suenaga, Mutou, Shibata, Itoh, Kigoshi & Yamada, 1996; Suenaga, Mutou, Shibata, Itoh, Fujita, Takada, Hayamizu, Takagi, Irifune, Kigoshi & Yamada, 2004).

Kisugi, Kamiya and Yamazaki (1989) obtained an antineoplastic glycoprotein from the body fluid of *Dolabella auricularia*. Kisugi, Ohye, Kamiya and Yamazaki (1992) found what they call

Photo Gallery of Animals Discussed in Text

1 *Cyphoma gibbosum*, a prosobranch gastropod that lives and feeds on the gorgonian *Gorgonia ventalina*. Photograph by Ernesto Mollo.
2 *Rictaxis punctocaelatus*, a primitive cephalaspidean from California. Photograph by Terrence Gosliner.
3 *Hydatina physis*, a tropical cephalaspidean. Photograph by Terrence Gosliner.
4 *Micromelo undata*, a tropical cephalaspidean. Photograph by Terrence Gosliner.
5 *Haminoea orbignyana*, a cephalaspidean of the family Haminoeidae. Photograph by Guido Villani.
6 *Haminoea orteai*. Photograph by Guido Villani.
7 *Haminoea cyanomarginata*. Photograph by Ernesto Mollo.
8 *Haminoea cymbalum*. Photograph by Ernesto Mollo.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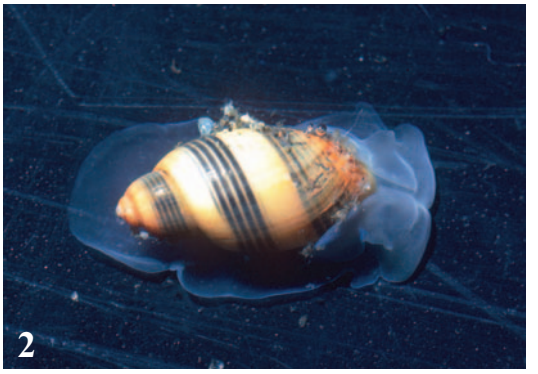
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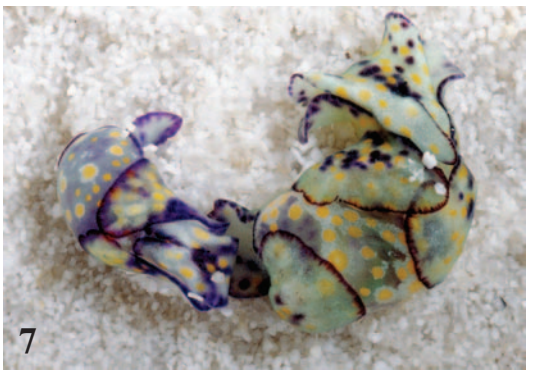
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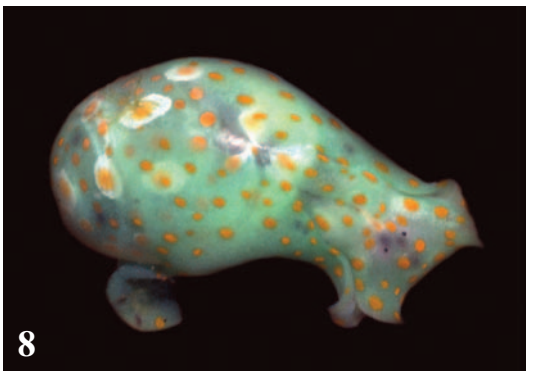
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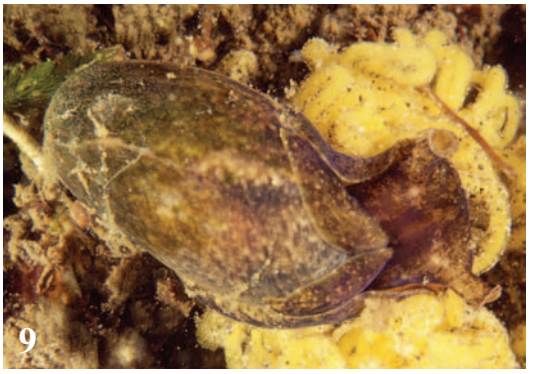
9 *Bulla striata*, with an egg mass. Photograph by Guido Villani.
10 *Smaragdinella calyculata*. Photograph by Terrence Gosliner.
11 *Scaphander japonicus*. Photograph by Terrence Gosliner.
12 *Philinopsis depicta*. Photograph by Guido Villani.
13 *Navanax inermis*. Photograph by Terrence Gosliner.
14 *Sagaminopteron ornatum*. Photograph by Ernesto Mollo.
15 *Sagaminopteron psychedelicum*. Photograph by Terrence Gosliner.
16 *Akera bullata*, a primitive anaspidean, swimming. Photograph by Terrence Gosliner.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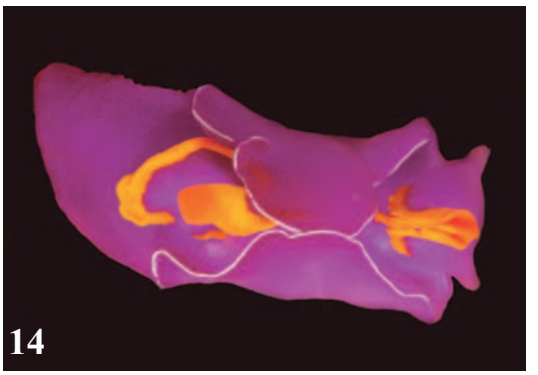
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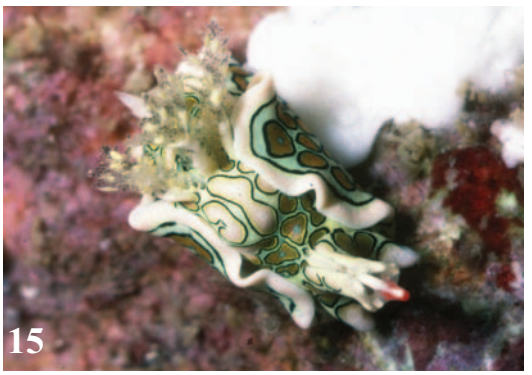
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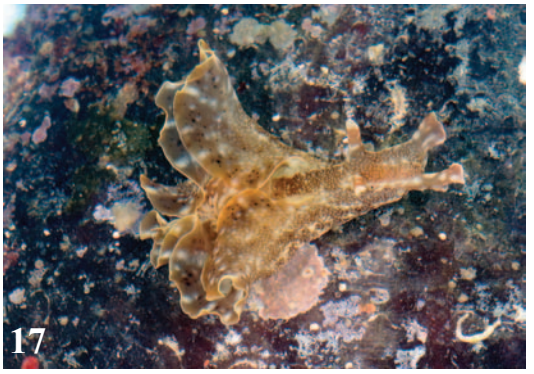
17 *Aplysia pulmonica*. Photograph by Terrence Gosliner.
18 *Aplysia parvula* swimming. Photograph by Guido Villani
19 *Dolabella auricularia*. Photograph by Ernesto Mollo.
20 *Stylocheilus striatus*. Photograph by Terrence Gosliner.
21 *Bursatella leachii*. Photograph by Terrence Gosliner.
22 *Dolabrifera dolabrifera*. Photograph by Terrence Gosliner.
23 *Syphonota geographica*. Photograph by Ernesto Mollo.
24 *Cylindrobulla* sp., a primitive sacoglossan. Photograph by Terrence Gosliner.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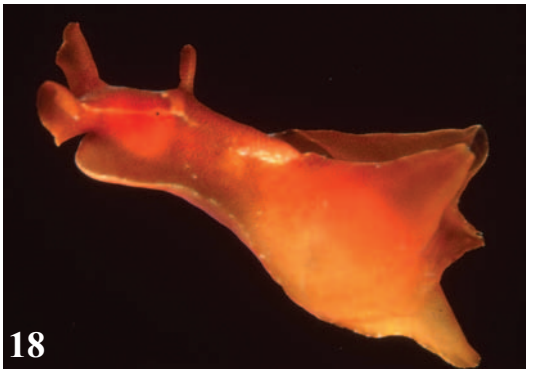
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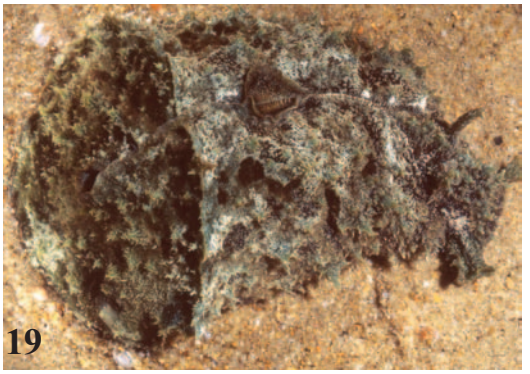
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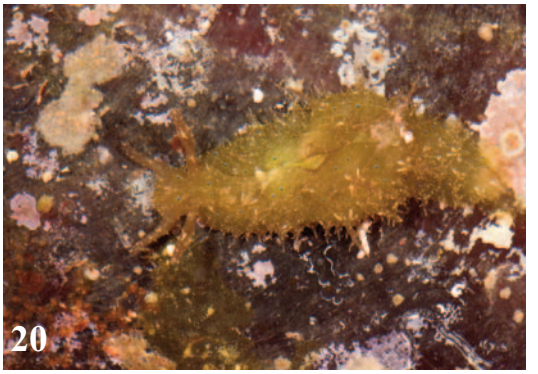
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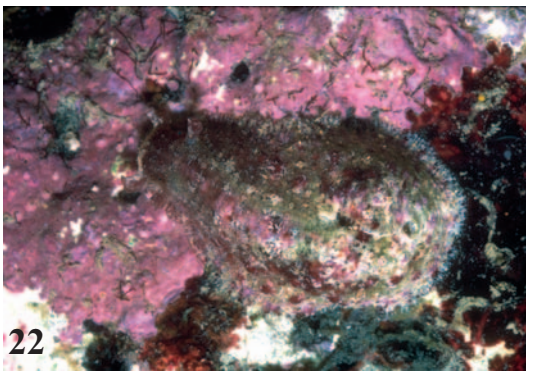
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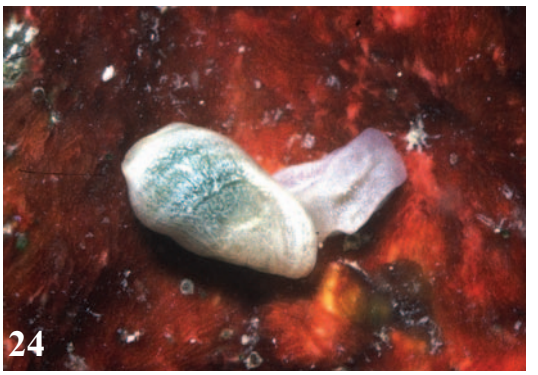
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25 *Ascobulla fragilis* (formerly *Cylindrobulla fragilis*). Photograph by Ernesto Mollo.
26 *Volvatella vigorouxi* exuding defensive secretion. Photograph by Ernesto Mollo.
27 *Oxynoe olivacea* on *Caulerpa prolifera*. Photograph by Ernesto Mollo.
28 *Lobiger serradifalci*. Photograph by Ernesto Mollo.
29 *Bosellia mimetica*, with eggs, on *Halimeda*. Photograph by Guido Villani.
30 *Elysia timida*. Photograph by Guido Villani.
31 *Elysia viridis*. Photograph by Guido Villani.
32 *Elysia subornata*. Potograph by Ernesto Mollo.


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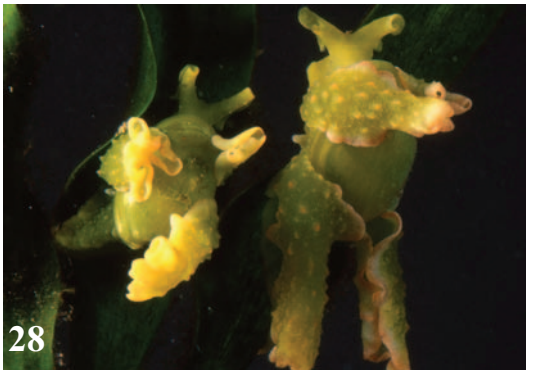
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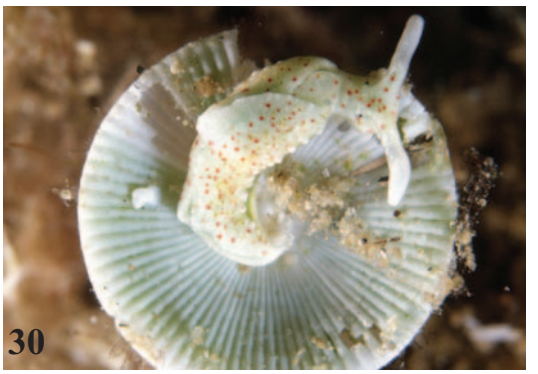
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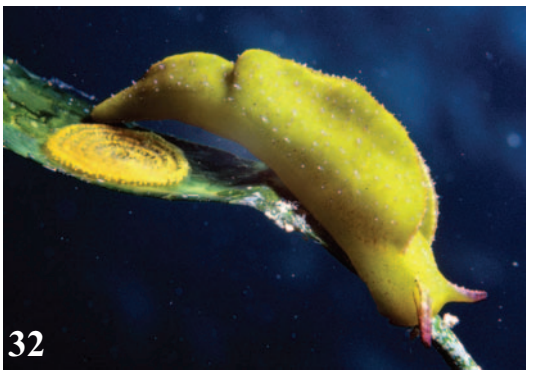
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33 *Plakobranchus* sp. Photograph by Ernesto Mollo.
34 *Thuridilla hopei*. Photograph by Guido Villani.
35 *Thuridilla gracilis*. Photograph by Terrence Gosliner.
36 *Costasiella ocellifera*. Photograph by Ernesto Mollo.
37 *Placida dendritica*. Photograph by Guido Villani.
38 *Ercolania funerea*. Photograph by Guido Villani.
39 *Cyerce cristallina*. Photograph by Ernesto Mollo.
40 *Calyphilla mediterranea*. Photograph by Ernesto Mollo.


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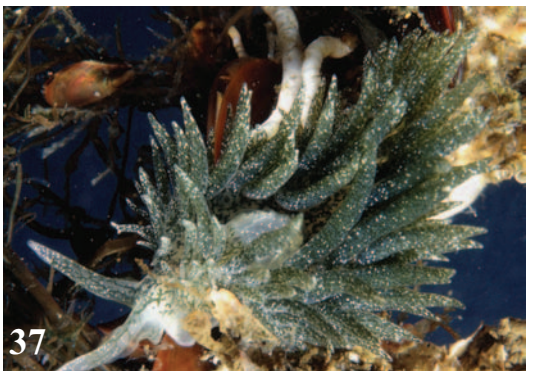
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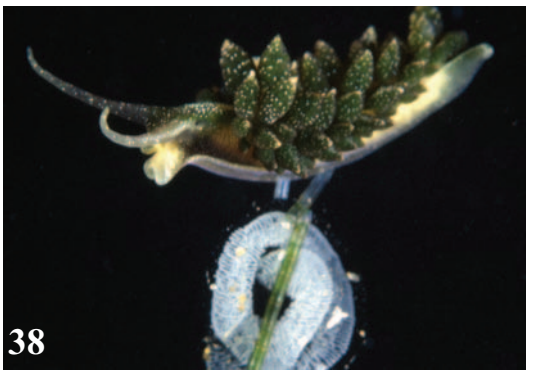
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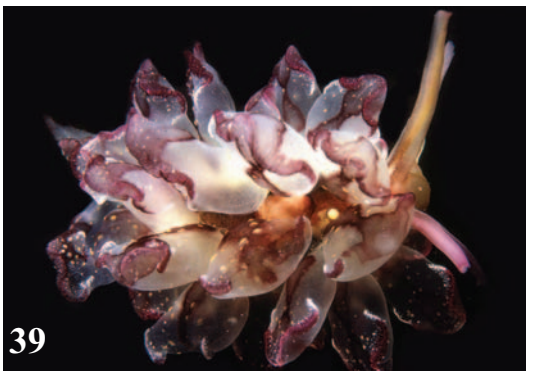
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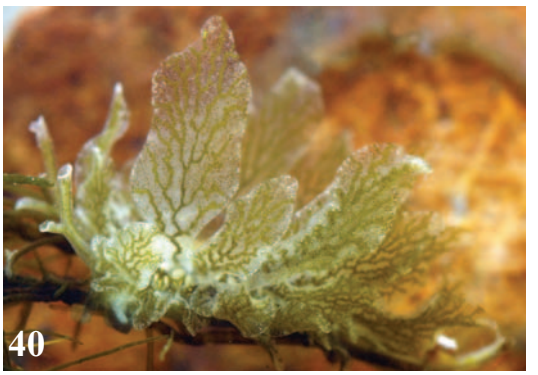
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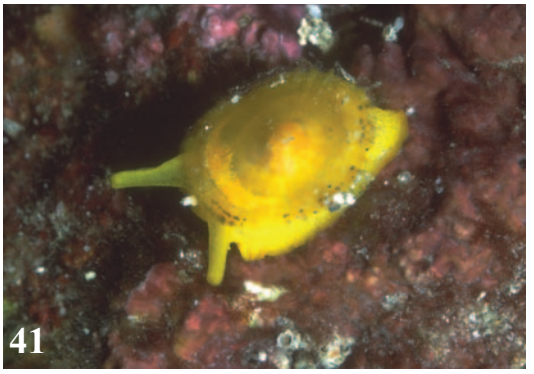
41 *Tylodina fungina*, a primitive notaspidean. Photograph by Terrence Gosliner.
42 *Umbraculum umbraculum*. Photograph by Guido Villani.
43 *Pleurobranchus testudinarius*. Photograph by Guido Villani.
44 *Pleurobranchus membranaceus*. Photograph by Guido Villani.
45 *Pleurobranchaea meckelii*, with eggs. Photograph by Guido Villani.
46 *Hexabranchus sanguineus*, a dorid nudibranch, swimming. Photograph by Ernesto Mollo.
47 *Polycera elegans* on bryozoans. Photograph by Guido Villani.
48 *Tambja capensis*. Photograph by Terrence Gosliner.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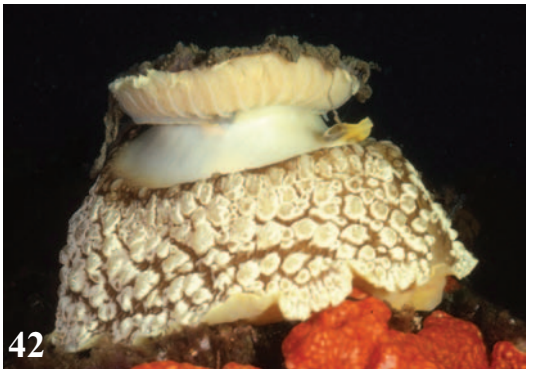
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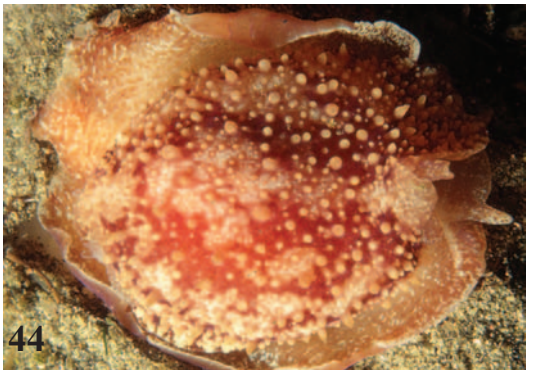
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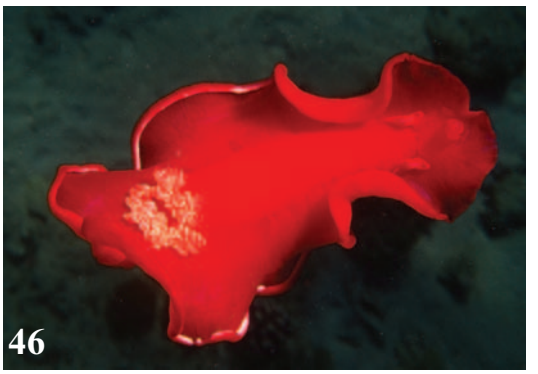
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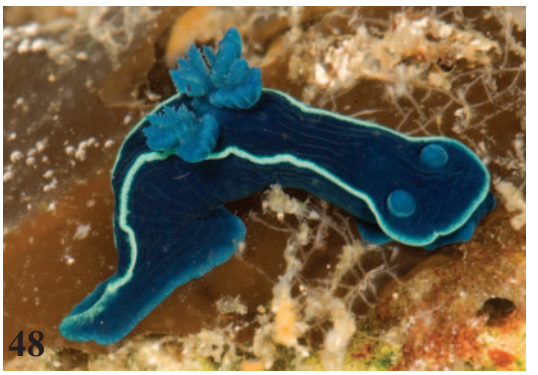
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49 *Tambja morosa*. Photograph by Terrence Gosliner.
50 *Tambja eilora*. Photograph by Terrence Gosliner.
51 *Roboastra tigris*. Photograph by Terrence Gosliner.
52 *Roboastra europaea*. Photograph by Guido Villani.
53 *Nembrotha kubaryana*. Photograph by Terrence Gosliner.
54 *Aegires gardineri*. Photograph by Terrence Gosliner.
55 *Acanthodoris nanaimoensis*, two views. Photographs by Terrence Gosliner
56 *Doriopsilla areolata*, with eggs. Photograph by Guido Villani.


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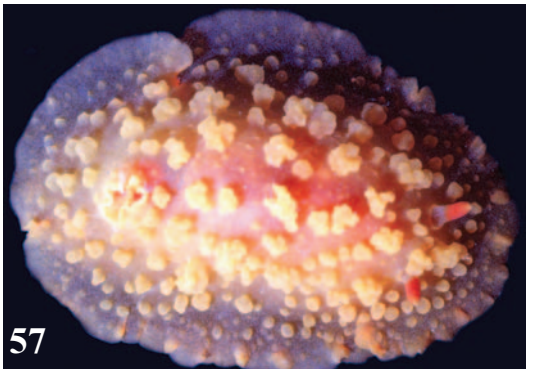
57 *Doriopsilla pelseneeri*. Photograph by Ernesto Mollo.
58 *Dendrodoris limbata*. Photograph by Ernesto Mollo.
59 *Chromodoris geometrica* (above) and *Phyllidiella pustulosa* (below). Photograph by Ernesto Mollo.
60 *Ovula ovum* (above), a prosobranch, mimics *Phyllidia carlsonhoffi* (below, left) and *P. pustulosa* (below, right). Photograph by Ernesto Mollo.
61 *Phyllidia varricosa*. Photograph by Terrence Gosliner.
62 *Phyllidia coelestis*. Photograph by Ernesto Mollo.
63 *Phyllidia flava*. Photograph by Guido Villani.
64 *Phyllidia ocellata*. Photograph by Ernesto Mollo.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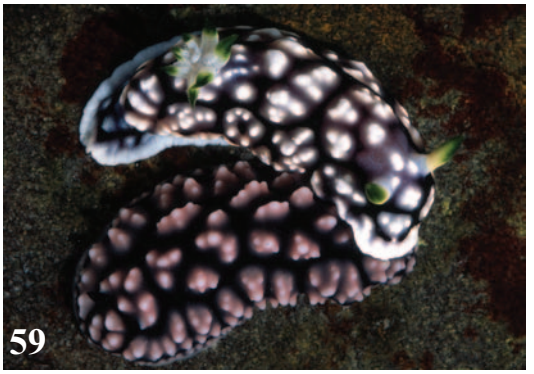
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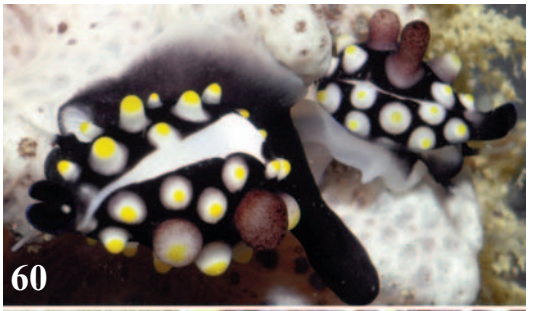
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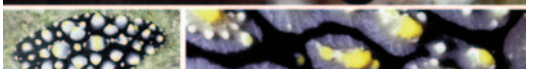


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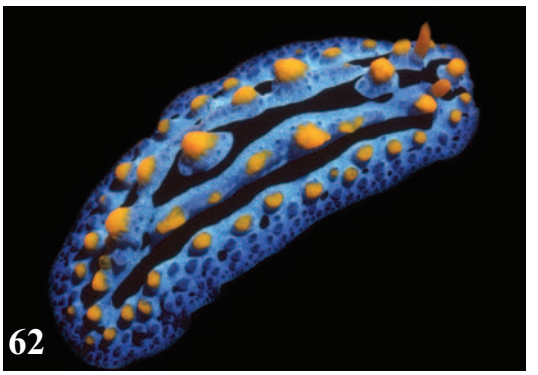
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65 *Phyllidella pustulosa*. Photograph by Terrence Gosliner.
66 *Reticulidia fungia*. Photograph by Terrence Gosliner.
67 *Cadlina pellucida*. Photograph by Ernesto Mollo.
68 *Glossodoris cincta*. Photograph by Ernesto Mollo.
69 *Glossodoris pallida*. Photograph by Ernesto Mollo.
70 *Glossodoris rufomarginata*. Photograph by Ernesto Mollo.
71 *Glossodoris averni*. Photograph by Ernesto Mollo.
72 *Glossodoris vespa*. Photograph by Ernesto Mollo.


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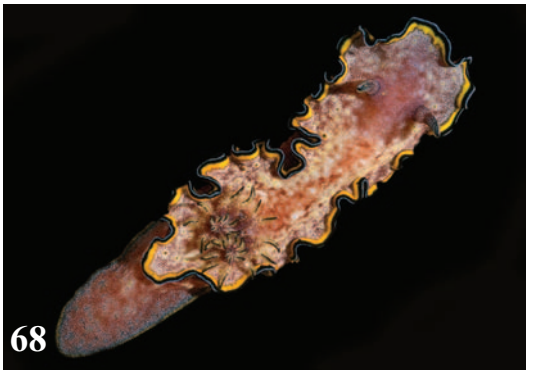
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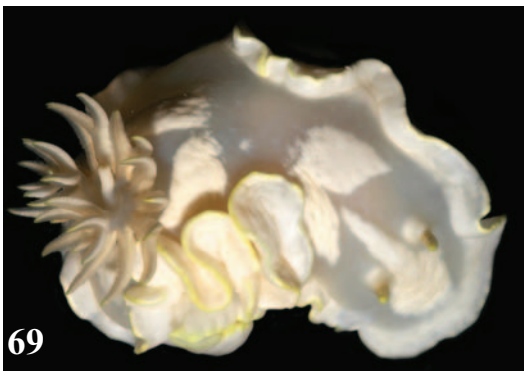
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73 *Chromodoris quadricolor*. Photograph by Ernesto Mollo.
74 *Chromodoris elisabethina*. Photograph by Terrence Gosliner.
75 *Chromodoris lochi*. Photograph by Terrence Gosliner.
76 *Chromodoris hamiltoni*. Photograph by Terrence Gosliner.
77 *Chromodoris luteorosea*. Photograph by Ernesto Mollo.
78 *Chromodoris marislae*. Photograph by Terrence Gosliner.
79 *Chromodoris geminus*. Photograph by Terrence Gosliner.
80 *Chromodoris annulata*. Photograph by Terrence Gosliner.


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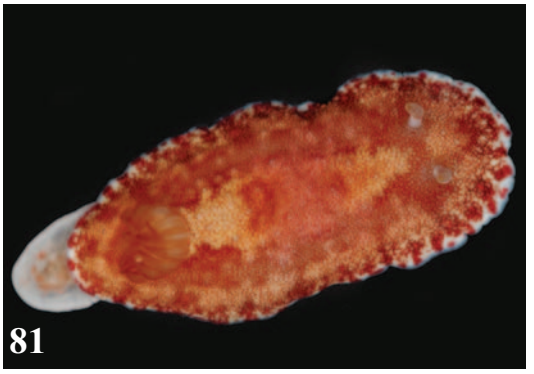
81 *Chromodoris reticulata*. Photograph by Ernesto Mollo.
82 *Chromodoris purpurea*. Photograph by Ernesto Mollo.
83 *Chromodoris macfarlandi*. Photograph by Terrence Gosliner.
84 *Chromodoris norrisi*. Photograph by Terrence Gosliner.
85 *Ceratosoma trilobatum*. Photograph by Ernesto Mollo.
86 *Ceratosoma gracillimum*. Photograph by Ernesto Mollo.
87 *Mexichromis macropus*. Photograph by Ernesto Mollo.
88 *Mexichromis porterae*. Photograph by Terrence Gosliner.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89 *Risbecia pulchella*. Photograph by Ernesto Mollo.
90 *Risbecia imperialis*. Photograph by Terrence Gosliner.
91 *Hypselodoris capensis*. Photograph by Terrence Gosliner.
92 *Hypselodoris maridadulus*. Photograph by Terrence Gosliner.
93 *Hypselodoris nigrostriata*. Photograph by Ernesto Mollo.
94 *Hypselodoris villafranca*. Photograph by Guido Villani.
95 *Hypselodoris orsini*. Photograph by Guido Villani.
96 *Hypselodoris fontandraui*. Photograph by Guido Villani.


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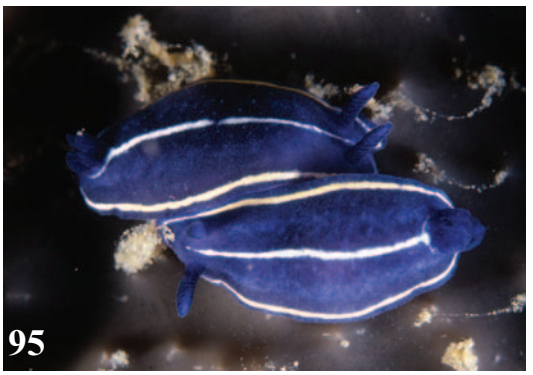
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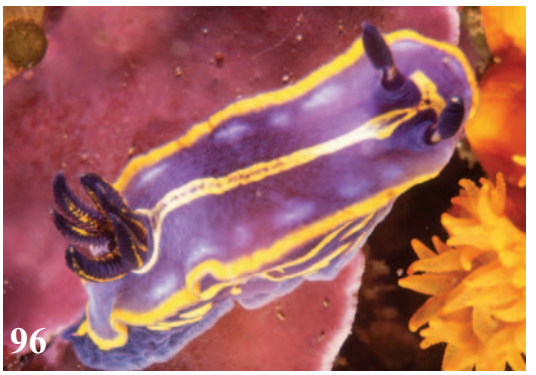
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97 *Hypselodoris picta*. Photograph by Ernesto Mollo.
98 *Hypseoldoris agassizii*. Photograph by Terrence Gosliner.
99 *Hypselodoris californiensis*. Photograph by Terrence Gosliner.
100 *Hypselodoris ghiselini*. Photograph by Terrence Gosliner.
101 *Aldisa smaragdina*. Photograph by Ernesto Mollo.
102 *Doris odhneri*. Photograph by Terrence Gosliner.
103 *Doris verrucosa*. Photograph by Guido Villani.
104 *Doris montereyensis*. Photograph by Terrence Gosliner.


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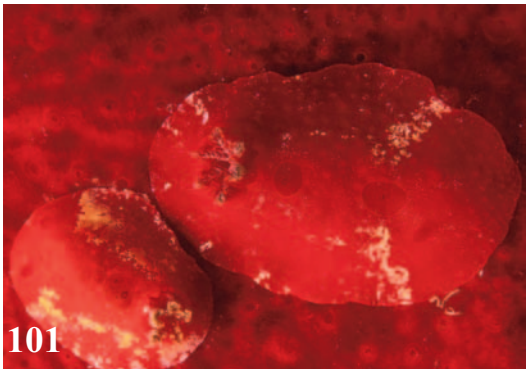
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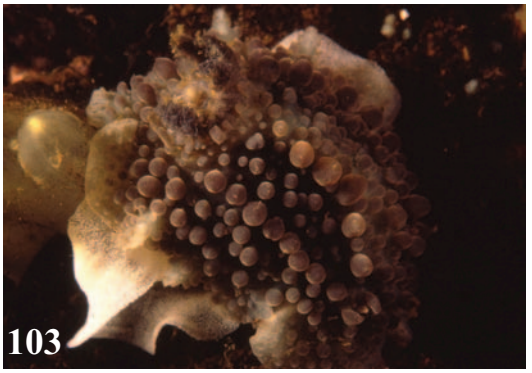
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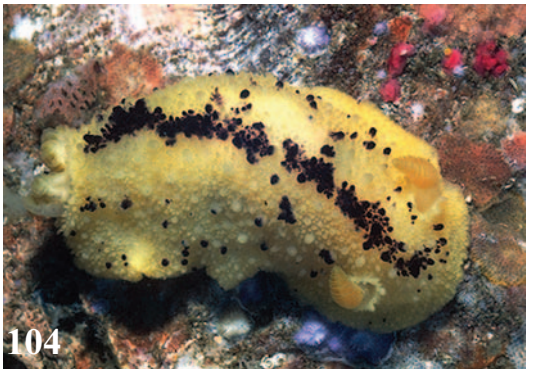
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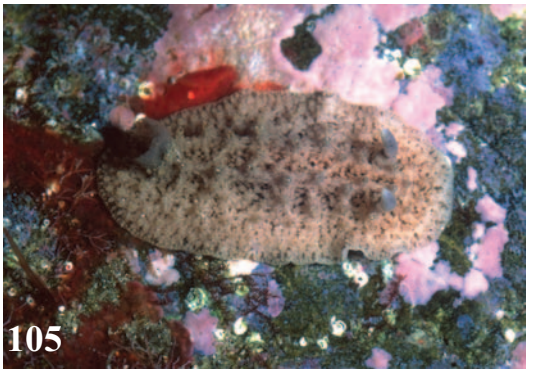
105 *Doris tanya*. Photograph by Terrence Gosliner.
106 *Diaulula sandiegensis*. Photograph by Terrence Gosliner.
107 *Jorunna funebris*. Photograph by Ernesto Mollo.
108 *Asteronotus caespitosus*. Photograph by Terrence Gosliner.
109 *Peltodoris nobilis*. Photograph by Terrence Gosliner.
110 *Peltodoris atromaculata*. Photograph by Guido Villani.
111 *Halgerda terramtuentsis*. Photograph by Terrence Gosliner.
112 *Halgerda willeyi*. Photograph by Terrence Gosliner.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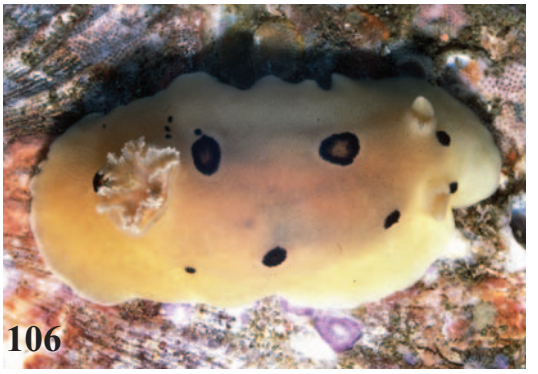
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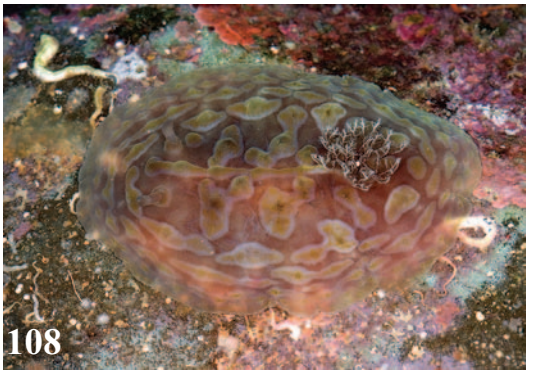
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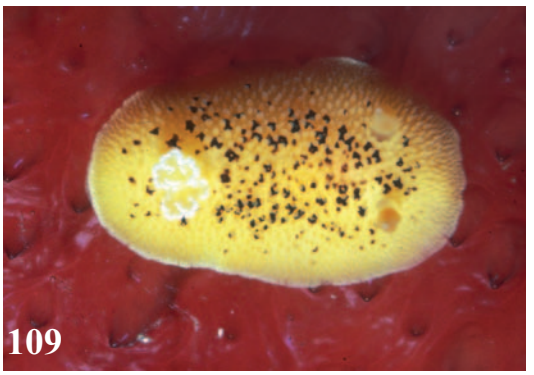
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113 *Tochuina tetraquetra*. Photograph by Terrence Gosliner.
114 *Tritonia hamnerorum* on a sea fan, with egg masses. Photograph by Terrence Gosliner.
115 *Tethys fimbria*. Photograph by Guido Villani.
116 *Melibe viridis*. Photograph by Ernesto Mollo.
117 *Dermatobranchus ornatus*. Photograph by Ernesto Mollo.
118 *Leminda millecra*. Photograph by Terrence Gosliner.
119 *Janolus cristatus*. Photograph by Guido Villani.
120 *Flabellina pedata* on a hydroid. Photograph by Ernesto Mollo.


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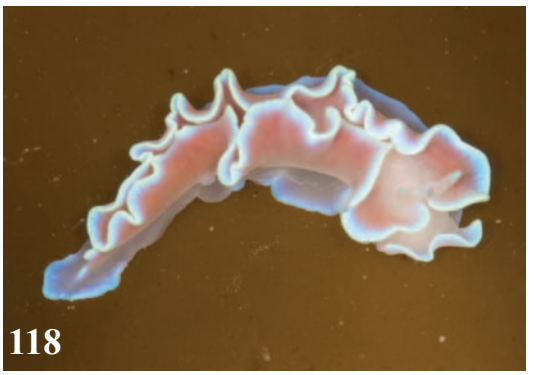
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121 *Flabellina affinis*. Photograph by Ernesto Mollo.
122 *Glaucus atlanticus*. Photograph by Terrence Gosliner.
123 *Phyllodesmium magnum*. Photograph by Ernesto Mollo.
124 *Cratena peregrina*. Photograph by Guido Villani.
125 *Flabellina lineata*. Photograph by Guido Villani.
126 *Godiva quadricolor*. Photograph by Guido Villani.
127 *Phestilla melanobrachia*. Photograph by Terrence Gosliner.
128 *Caloria indica*. Photograph by Ernesto Mollo.


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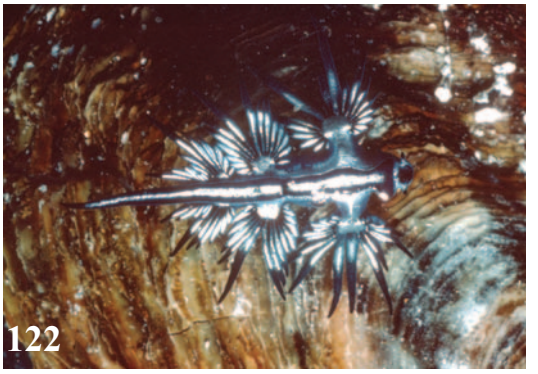
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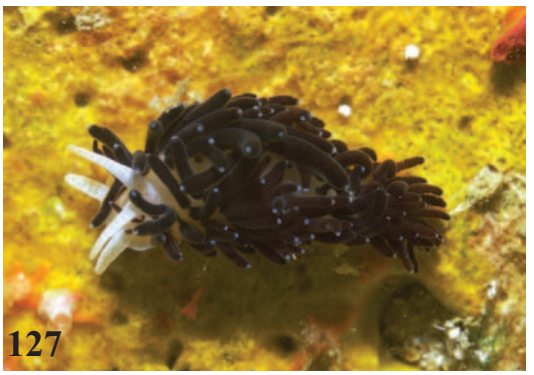
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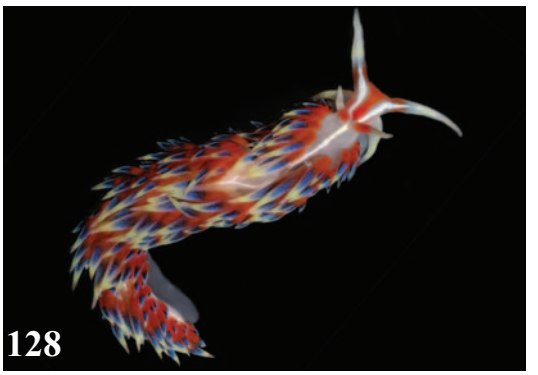
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an antibacterial protein from the albumen gland (part of the reproductive system that deposits nutriment around the eggs) of the same species. These of course are produced by the animals themselves.

Turning to the Notarchinae, there are no data on secondary metabolites in *Notarchus* itself. *Stylocheilus striatus* (Quoy & Gaimard, 1832) (previously known as *S. longicaudus*), shown in **Photo 20**, provides an interesting example of an herbivore that has switched from algae to cyanobacteria as a source of food and defensive metabolites. This animal, which is widely distributed in the Pacific, often occurs in large aggregations. Kato and Scheuer (1974, 1975) described two polyethers from it, and gave them the misleading names aplysiatoxin (**Atlas 59**) and debromoaplysiatoxin (**Atlas 60**). Rose, Scheuer, Springer & Clardy (1978) found these slugs feeding on the red alga *Acanthophera spiculifera* and the cyanobacterium *Lyngbya majuscula*, and described a non-toxic amide from it, stylocheilamide. The structure was revised as being identical to acetyl malyngamide (**Atlas 605**) detected in *Lyngbia majuscula* by Todd and Gerwick (1995). *Stylocheilus striatus* has been found to contain complex proline esters (Gallimore, Galario, Lacy, Zhu & Scheuer, 2000) and various alkaloids (Gallimore & Scheuer, 2000).

Paul and Pennings (1991) found that *Stylocheilus striatus* feeds on a cyanobacterium and derives secondary metabolites from it, although it does feed on true algae as well. Feeding preference experiments indicated that they favor the cyanobacterium and grow better on it. Deterrence experiments using fish showed that the wrasse, *Thalassoma hardwicki*, although somewhat slowed down, finally ate it. Pennings and Paul (1993) identified the cyanobacterium as *Microcoleus lyngyaceus*, and observed that the slug sequesters malyngamide A (**Atlas 607**) and B (**Atlas 610**), converting some of the malyngamide B into the acetate (**Atlas 611**). They found that malyngamide would deter feeding, as would some other metabolites. Further deterrency studies were reported by the same group (Pennings & Paul, 1993; Pennings, Pablo, Paul & Duffy, 1994; Pennings, Weiss & Paul, 1996; Nagle, Camacho and Paul (1998). The results indicated that earlier studies of deterrency were flawed because of the concentrations used. Perhaps their most interesting result was that malyngamides and majusculamides acted as feeding attractants at low concentrations and as powerful deterrents at high concentrations. There are similar cases in other animals, including opisthobranchs. Pennings, Nastich, and Paul (2001) raised *Stylocheilus* on artificial diets, with and without metabolites, and fed them to fish in the wild; they concluded that there was little evidence for a protective role. But, as with the skepticism about *Aplysia*, such negative results are hard to interpret. In another study, crude extracts of *Lyngbya majuscula* were tested against various small predators (Capper, Cruz-Rivera, Paul & Tibbetts, 2006). *Stylocheilus* was stimulated to feed by the extracts, whereas others, including the cephalaspidean *Diniatys dentifer*, were repelled. (See also Capper, Tibbetts, O'Neill and Shaw, 2005).

The other genus in the Notarchinae for which we have data on secondary metabolites is *Bursatella* (*B. leachii,* **Photo 21**). It contains a diol nitrile alkaloid called bursatellin (**Atlas 619**), which is structurally related to the antibiotic chloramphenicol (Gopichand & Schmitz, 1980). The originally proposed structure has been somewhat revised (Cimino, Gavagnin, Sodano, Spinella, Strazzullo, Schmitz & Gopichand, 1987). There are supposedly three subspecies: *Bursatella leachii plei* from Puerto Rico, *B. leachii leachii* from the Tyrrhenian Sea, and *B. leachii savignyana* from the Adriatic. The mantles of the latter two subspecies were extracted separately (Cimino, Gavagnin, Sodano, Spinella, Strazzullo, Schmitz & Gopichand, 1987). It was found that bursatellin occurred in the mantle tissues of both subspecies, but only *B. leachii savignyana* had it in the digestive glands. Surprisingly, the two subspecies contained two enantiomers of bursatellin, the + isomer in *B. leachi leachi* and the - form in *B. leachii savignyana*.

Specimens of *Bursatella leachii* from New Zealand reveal that it contains other very unusual

metabolites. It is known to feed on cyanobacteria and to contain their typical metabolites and the alkaloids dactylamides A and B (**Atlas 601, 602**) (Appleton, Sewell, Berridge, & Copp, 2002; , Appleton & Copp, 2005). Animals from Thailand were found to contain a chlorinated alkaloid (**Atlas 594**) (Suntornchashwei, Chaichit, Isobe & Suboriux, 2005). Four antitumor substances were identified in extracts of an organism from the South China Sea, reported as "*Notarchus* (*Bursatella*) *leachii cirrosus* (Stimpson 1855)" (Lin, Zhang, Yi, Shen, Guo & Shao, 2001; Lin, Tang, Liu, Zhang, Shen, Hua & Wu, 2002). Two of these are chlorinated monoterpene esters, whereas the others are steroids.

The Dolabriferinae include the genera *Petalifera*, *Phyllaplysia*, and *Dolabrifera*, in which there has been a change in diet. These animals graze on diatoms, often those that live epiphytically on marine plants such as seaweeds and marine "grasses". Secondary metabolites have been found in only one species of these anaspideans (Ciavatta, Gavagnin, Puliti & Cimino, 1996). *Dolabrifera dolabrifera*, shown in **Photo 22**, contains the polypropionate dolabriferol (**Atlas 119**). This is a non-contiguous polypropionate, which is most unusual. Such compounds have been found only in some pulmonates, *Pleurobranchus membranaceus* and *Micromelo undata*. Dolabriferol does not occur in the digestive gland, but is limited to the integument. Therefore it seems quite likely that, like other propionates in herbivorous opisthobranchs and pulmonates, it is biosynthesized *de novo*, although this has not been confirmed experimentally. It provides a nice example of *de novo* synthesis making an appearance when there has been a change in diet and exogenous defensive metabolites are no longer available. This has happened well within the group: *Dolabrifera* is not very closely related to *Dolabella*, which is the only other anaspidean in which polypropionates have been found. However, the polypropionates from *Dolabella* were from the internal organs of the animal. They are not in the appropriate place, nor are they in high enough concentration, to defend the animal from predators. Furthermore *Dolabella* is very well protected by exogenous metabolites. The hypothesis that the propionates derive from sacoglossans inadvertently ingested along with algae seems dreadfully *ad hoc*. The obvious alternative is that polypropionates are more widespread than the lack of evidence suggests. A concerted effort to find them might yield valuable results.

The pteropods are pelagic opisthobranchs, so called after the wing-like extensions of the foot with which the animals swim. Two separate orders are recognized — Thecosomata and Gymnosomata — so-called because the body is covered by a shell in the former, whereas the latter are "naked." They are quite different in both structure and way of life. Thecosomes are herbivores that feed with mucous nets. Gymnosomes are active predators and eat small animals, especially thecosomes. Pteropods were formerly treated as a single group, but the Belgian malacologist Paul Pelseneer (1899, 1906) decided that the Gymnosomata are derived from Anaspidea, whereas the Thecosomata are derived from Cephalaspidea. It was not clear which group of cephalaspideans was supposed to have given rise to the thecosomes. Furthermore, anaspideans themselves are modified cephalaspideans. The possibility that both groups of pteropods are derived from a stock closely related to Anaspidea was maintained by various authors, including one of us (Ghiselin, 1996b). A molecular study based on 28S ribosomal RNA sequence data has given strong support to that relationship (Dayrat, Tillier, Lecointre & Tillier, 2001). Additional molecular evidence leads to the same conclusion (Klussmann-Kolb & Dinapoli, 2006; Malaquias, Mackenzie-Dobbs, Gosliner & Reid, 2009).

The issue of pteropod relationships is somewhat obscured by the tradition of leaving them out of consideration in many discussions of the opisthobranchs in general. For our purposes it is not a crucial matter. There are no published reports of defensive metabolites among the Thecosomata. In Gymnosomata only one species, *Clione antarctica*, is known to have defensive metabolites. It con-

tains pteroenone (**Atlas 83**), a polypropionate (Yoshida, Bryan, Baker & McClintock, 1995; McClintock & Baker, 1998). Pteroenone does not occur in *Limacina*, the thecosome upon which *C. antarctica* feeds, making it seem unlikely that the propionate is derived from food (Bryan, Yoshida, McClintock & Baker, 1995). An amphipod crustacean protects itself by a kind of parasitism, carrying the pteropod about (McClintock & Janssen, 1990). Once again, we find that the use of defensive polypropionates accompanies a major shift in diet and habitat.

Chapter VIII

Sacoglossa

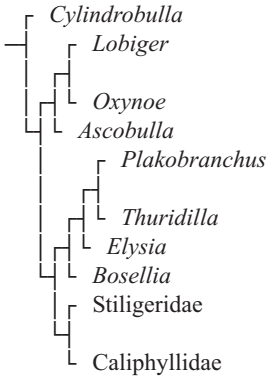
The sacoglossans, or ascoglossans, are remarkably specialized herbivores. The radula has been modified, so that it has a single row of teeth, allowing the animals to pierce the cells of algae and suck out the soft and nutritious contents. The worn-out teeth are deposited in a sac, or ascus, hence the names. Sometimes called "sap-sucking slugs," they have been compared to aphids and other insects that feed on terrestrial plants. This comparison is in some ways apt, but there are many differences too. In the slugs, as in most other benthic marine animals, it is the larval stage that disperses and locates the food, whereas in the aphids (and terrestrial animals in general) it is the adult (Duffy, Paul, Renaud & Fenical, 1990). Their chemical defense has been treated from an evolutionary point of view by Cimino and Ghiselin (1998) and by Marin and Ros (2004), and from an ecological one by Cimino, Fontana, and Gavagnin (1999).

In some lineages within the group a remarkable way of exploiting the algae upon which the animals feed has evolved (Muscatine & Greene, 1973; Hind & Smith, 1974; Clark & Busacca, 1978; Ros & Marin, 1991; Rumpho, Sumner & Manhart, 2000; Wägele & Johansen, 2001). The chloroplasts are separated from the remaining part of the algal material that has been ingested and then kept alive and functioning inside cells that line the diverticula of the slugs' digestive systems. Evidently the use of chloroplasts from food as captive photosynthetic apparatus was preceded by an earlier stage when their green color provided for camouflage (Clark, Jensen & Stirts, 1990). Algal chloroplasts evolved from free-living, photosynthetic bacteria. The bacteria got incorporated within the cells of eukaryotic organisms, allowing them to become photosynthetic and derive their energy from sunlight. The chloroplasts are much simplified organisms, and highly dependent upon the rest of the plant cell for support. Chloroplasts cannot be kept alive in culture for very long because they are dependent upon materials from the nucleus of the plant. Some sacoglossans can keep the chloroplasts alive and functioning for months on end. It was proposed that there has been a transfer of genes from the nucleus of the plant to that of the slugs (Pierce, Massey, Hanten & Curtis, 2003). Subsequently it has been shown that in *Elysia chlorotica* functional algal nuclear genes are indeed present in the nucleus of the slug and that they are transmitted to the slug's offspring (Pierce, Curtis, Hanten, Boerner & Schwartz, 2007; Rumpho, Worful, Lee, Kannan & Tyler, 2008). The sacoglossans are ectoparasites of the endosymbiotic chloroplasts. It has been speculated that the relationship is a mutualistic one, and that the algae, though not the chloroplasts, derive some kind of benefit from it (Ros & Marin, 1991). The evidence presented suggests, at most, that the harm is not as great as it seems.

When the nutritive symbiosis is well developed, the slugs become independent of any other source of energy. *Elysia crispata* (formerly *Tridachia crispata*) is said to stop feeding when it reaches a length of around 13 mm, although it grows to a considerably larger size (Thompson & Jarman, 1989). *Plakobranchus* (**Photo 33**), which can often be found basking in the sun in shallow waters in the tropics, has been considerably modified in adaptation to this way of life. The dorsal

surface of this animal is bright green, thanks to the abundant concentration of chloroplasts in branches of the digestive gland. The sides of the foot can be extended over the green area, and when this is done the animal is relatively inconspicuous on sandy bottoms. Microscope studies showed very little evidence of algal material in the gut, indicating that the chloroplasts are retained for a considerable period (Eiuchi, 2005). Young ones raised on sterile sand without food were found to continue to grow and reproduce, thus relying entirely upon the symbionts for nutrition (Marilyn Switzer-Dunlap, personal communication).

The following diagram suggests the relationships among sacoglossans:



The genealogical relationships of sacoglossans are fairly well understood, thanks largely to the contributions of Gosliner (1995) and Jensen (1996, 1997, 1997). As mentioned earlier, efforts to place them in the tree of the opisthobranchs as a whole have given equivocal results, but their having a close relationship to anaspideans and perhaps to cephalaspideans of the family Diaphanidae has been maintained by Ghiselin (1966) and Jensen (1996) as well as some earlier authors. The group would therefore have arisen from an herbivorous ancestry that became specialized for feeding suctorially on siphonaceous algae, which are vulnerable because their cells are continuous. Which alga was the ancestor has been the subject of some uncertain speculation. Because the genus *Caulerpa* is the host of many sacoglossans, it has been a popular candidate. However, it has seemed likely that it was some other member of its family, such as *Udotea*.

The most primitive genus within the Sacoglossa is *Cylindrobulla*, an unidentified species of which is shown in **Photo 24** (see Jensen, 1989). Although some species of this genus feed on *Caulerpa*, some are known to feed on *Halimeda* as well. *Cylindrobulla* may reasonably be assumed to have branched off before all other sacoglossans, because it lacks the ascus from which the group derives its name. Presence of an ascus then is a shared, derived character indicating that the rest of the sacoglossans form a natural group. Many sacoglossans have shells, but even more of them have evolved into slugs. At one time it was generally assumed that the transition from snail to slug occurred in several independent lineages. However, this was just a guess, and no evidence was presented to connect the slug-like forms to two different shelled lineages. Therefore we accept the view that the shelled forms other than *Cylindrobulla* form a natural group and that the shell-less forms are its likewise monophyletic sister group. We will now treat these two groups, beginning with the shelled ones.

All of the "higher" shelled sacoglossans feed upon algae of the genus *Caulerpa* and live in close association with it. None of them have been found to utilize photosynthesizing chloroplasts as a source of energy. Among these the genus *Ascobulla* is the most primitive in structure and habits. It looks very much like *Cylindrobulla* externally but it does have the characteristic radula

and ascus. It still lives and feeds buried in the substrate. **Photo 26** shows another sacoglossan, *Volvatella vigourouxii*, exuding metabolites from the posterior end of the shell. *Ascobulla fragilis*, shown in **Photo 25**, feeds upon *Caulerpa prolifera* and utilizes the algal secondary metabolite in its own defense. The plant contains a sesquiterpenoid, caulerpenyne (**Atlas 206**), which has already been discussed from a chemical point of view. It sequesters the caulerpenyne and modifies it to form the more toxic oxytoxin-1 (**Atlas 207**) and the still more toxic oxytoxin-2 (**Atlas 208**) (Gavagnin, Marin, Castelluccio, Villani & Cimino, 1994). The oxytoxin-2 is secreted in mucus when the animal is molested by a predator. It has been proved, as we noted above, that some selective lipases are active in *Oxynoe olivacea*.

In *Oxynoe* and *Lobiger*, the shell is somewhat more reduced and the animals live on the exposed parts of *Caulerpa*, where they are well camouflaged, as can be seen in **Photo 27**, in which metabolites are being discharged by a specimen of *Oxynoe olivacea*. This animal feeds upon *Caulerpa prolifera*, and, again, modifies the caulerpenyne into oxytoxin-1 and oxytoxin-2. These defensive compounds are produced by the slug itself, by enzymatic hydrolysis of the algal metabolite (Cutignano, Notti, d'Ippolito, Domènech Coll, Cimino & Fontana, 2004). When attacked by a predator the animal waves the back end of the foot, which it can break off (autotomize) and then regenerate, much as happens to the tails in some lizards (Stamm, 1968; Lewin, 1970; Warmke & Almovódar, 1972). Note that the animal has several means of protection, of which autotomy is the most expensive. In *Lobiger* the shell is somewhat more reduced and the animal has large parapodial lobes. These are autotomized when the animal is molested (Gonor, 1961; Stamm, 1968). *Lobiger serradifalci*, shown in **Photo 28**, feeds on *Caulerpa prolifera*. It was found to contain oxytoxin-1 and oxytoxin-2 but no caulerpenyne.

The above Mediterranean shelled sacoglossans have relatives in the Caribbean. A comparative study showed that *Ascobulla ulla* (which feeds on *Caulerpa fastigiata*), *Oxynoe antillarum* (which feeds on an unidentified *Caulerpa*), and *Lobiger souberveii* (which feeds on *Caulerpa racemosa*), have the same basic patterns of metabolite utilization as do their Mediterranean counterparts (Gavagnin, Marin, Castellucio, Villani & Cimino, 1994; Gavagnin, Mollo, Montanaro, Ortea & Cimino, 2000). In *Ascobulla ulla* there are metabolites, ascobullin A and B (**Atlas 209, 210**), obtained by oxidation and reduction of the more toxic caulerpenyne (**Atlas 206**). This suggests an unspecialized condition relative to other sacoglossans. Volvatellin (**Atlas 211**) from an Indian species has also been recorded (Fontana, Ciavatta, Mollo, Naik, Wahidulla, D'Sousa & Cimino, 1999). There are no published reports on secondary metabolites from the "bivalved gastropods" mentioned earlier in this chapter.

Passing now to the remainder of the Sacoglossa, which are all forms in which a shell is absent except in the larva, they seem to consist of two natural groups, which can easily be separated on the basis of external anatomy. The first of these is the family Plakobranchidae (or Elysiidae). In these animals the body is rather leaf-shaped and the edge of the foot extends as parapodia, rather like those of anaspideans. The second consists of two families: Caliphyllidae and Stiligeridae. Here there are dorsal processes called cerata (singular ceras) that occur in two series down the animal's back. Similar structures, also called cerata, occur in some nudibranchs, and the superficial resemblance was once interpreted to indicate a close relationship.

Gosliner (1995) has identified three clades within the Plakobranchidae. The first of these consists of the genus *Bosellia*. It is the sister group of a clade consisting of the other two, which are *Elysia* in a broad sense on the one hand and *Plakobranchus* + *Thuridilla* on the other. Within the group there have been noteworthy shifts from one food item to another, but usually within the same order of plants (Bryopsidales = Caulerpales = Codiales = Siphonales). The exact evolutionary sequence of algae used as food is uncertain. Jensen (1993a, 1993b, 1993c, 1996b) has suggested

that the initial food was not *Caulerpa*, but perhaps some other siphonaceous algal genus such as *Halimeda*, *Udotea* or *Chlorodesmis*.

The genus *Bosellia*, which makes up the first branch of the Elysiidae, is not a good model for the common ancestor of the family. It is highly modified in many respects, and even has an unusually low chromosome number. Instead of crawling about with its parapodia somewhat upraised, it lies flat on the surface of the alga on which it feeds with the parapodia outstretched, as can be seen in **Photo 29**, which shows the animal next to its eggs. This alga is *Halimeda*, which is protected as a result of being highly calcified as well as by having secondary metabolites. A good example is *Bosellia mimetica*, which lives on *Halimeda tuna* (Gavagnin, Marin, Mollo, Crispino, Villani & Cimino, 1994). The defensive metabolite is the diterpenoid halimedatrial (**Atlas 377**), which is formed by activation of halimedatetracetate (**Atlas 376**) (Paul & Fenical, 1983, 1984; Paul & Van Alstyne, 1988, 1988, 1992). This diterpenoid metabolite differs from the sesquiterpenoid caulerpenyne (**Atlas 206**) of *Caulerpa* in having 20 rather than 15 carbon atoms, but otherwise it is quite similar. The active sites are identical, and in both a protected conjugated 1,4-dialdehyde gets activated by removal of the acetate group. Shifting from one of these metabolites to another would not be very difficult, but it is not obvious which came first in evolutionary history.

In the genus *Elysia* there are a number of species that feed upon *Caulerpa* and its close relatives (Gavagnin, Mollo, Montanaro, Ortea & Cimino, 2000). These generally utilize metabolites from the algae defensively. *Elysia subornata* lives on *Caulerpa prolifera*, which contains caulerpenyne. The slug contains both caulerpenyne and oxytoxin-1, implying that, like shelled sacoglossans, it can modify the algal metabolites (Gavagnin, Mollo, Montarno, Ortea & Cimino, 2000). The same metabolites are also found in *Elysia patina*, and *E. nisbeti*, which derive them from unidentified *Caulerpa*. Specimens of what has been tentatively identified as *Elysia expansa* were found to contain large amounts of caulerpenyne, together with minor amounts of dihydrocaulerpenyne (**Atlas 213**) and expansinol (**Atlas 214**) suggesting that the animal can reduce metabolites derived from the alga (Ciavatta, Lopez Gresa, Gavagnin, Manzo, Mollo, D'Souza & Cimino, 2006), showing some analogies with *Ascobulla ulla*. Two species feed on *Halimeda* and utilize its terpenoids. *Elysia pusilla* (formerly called *E. halimedae*) derives halimedatetraacetate and halimedatrial from *Halimeda macroloba* (Paul & Van Alstyne, 1988). *Elysia tuca* gets halimedatetraacetate (**Atlas 376**) from *Halimeda incrassata* (Gavagnin, Mollo, Montanaro, Ortea & Cimino, 2000). *Elysia translucens* feeds upon *Udotea* from which it derives the linear diterpenoid udoteal (**Atlas 375**) (Gavagnin, Marin, Mollo, Crispino, Villani & Cimino, 1994). This metabolite is closely related to halimedatetraacetate (**Atlas 376**) (Paul, Sun & Fenical, 1982; Paul & Fenical, 1984).

Several species of *Elysia* feed upon other algae and have quite different chemical defense. *Elysia rufescens* obtains toxic polypeptides (**Atlas 647-654**) from the *Bryopsis* upon which it feeds (Hamann & Scheuer 1993; Hamann, Otto, Scheuer, & Dunbar, 1996; Becerro, Goetz, Paul & Scheuer, 2001). Two acyclic kahalalides have also been reported from this species (Goetz, Nakao & Scheuer 1997). These are the first of a long series of similar compounds that have been described. A depsipeptide (**Atlas 657**) has been recorded from *Elysia ornata* and an unidentified *Bryopsis* (Horgen, de los Santos, Goetz, Sakamoto, Kan, Nagai & Scheuer, 2000). Further kahalalides (**Atlas 655, 666**) have been reported from *Elysia grandifolia* (Ashour, Edrada, Ebel, Wray, Wätgen, Padmakumar, Müller, Lin & Proksch, 2006). In what is said to be the same species two new ones (**Atlas 658, 659**) were found together with three others, but only one of these was recovered from *Bryopsis plumosa*, upon which it feeds (Tilvi & Naik, 2007). The two groups of investigators named two slightly different cyclic peptides in the same manner, so that kahalalides *R* and *S* both refer to two different molecules. The kahalalides are produced by bacteria, and the metabolite content of the food would seem to be variable.

Other sacoglossans biosynthesize polypropionates *de novo* instead of deriving metabolites from food. A good example is *Elysia timida* (**Photo 30**), which feeds selectively upon *Acetabularia acetabulum*, and contains three different propionates (**Atlas 123, 126, 131**), none of which occur in the alga (Gavagnin, Spinella, Castelluccio, Cimino & Marin, 1994). This species has been studied experimentally in order to determine its effectiveness as a feeding deterrent. Using model sacoglossans, fish were taught to associate color patterns with particular metabolites (Giminez-Casalduero, Muniain & Garcia-Charton, 2002). *Elysia viridis* (**Photo 31**) feeds upon *Codium vermiliara* and synthesizes the polypropionate (+)elysione (**Atlas 130**) (Gavagnin, Marin, Mollo, Crispino, Villani & Cimino, 1994). Elysione is also produced by *Elysia chlorotica* (Dawe & Wright, 1986). It feeds on *Cladophora* (Jensen, 1980).

Closely related to *Elysia timida* are two species of *Elysia* formerly called *Tridachia crispata* and *Tridachiella diomedea* (Gosliner, 1995). They produce unusual propionate-derived γ-pyrones such as tridachiapyrone A to F (**Atlas 134-141**). It has been suggested that tridachiapyrone A is the enantiomer of (+)elysione (Dawe & Wright, 1986). (See also Ireland, Faulkner, Solheim & Clardy, 1978; Ireland & Faulkner, 1981; Ksebati & Schmitz, 1985). Absolute configuration has recently been suggested by synthesis (Bourdron, Commeiras, Audran, Vanthuyne, Hubaud & Parrain, 2007). These are animals that photosynthesize using captive chloroplasts. The defensive role of these metabolites has been denied, though largely on the basis of negative evidence. But, supposing that they are not protective, what are they doing? Ireland and Scheuer (1979) proposed that they act as sunscreens, protecting the photosynthetic apparatus. This hypothesis is quite reasonable, and the photoreactivity of such compounds has been extensively studied. But there is still no compelling evidence in its favor.

More recent studies have revealed that the polypropionates serve as traps for oxygen and are photosensitized by singlet oxygen. This development has stimulated a considerable amount of new research on their photochemistry. The γ-pyrones have greater photosensitizing capability than do the α-pyrones and slugs evidently produce them preferentially. The photosensitized compounds, which are hydroperoxides and endoperoxides, are thought to have irritant properties, and therefore it has been suggested that these are the active agents in chemical defense (Zuidema & Jones, 2006). Recently a series of rigorous biosynthetic experiments with stable isotopes (^{13}C) led to unambiguous proof of the biosynthesis of (+)elysione in *Elysia viridis* (Cutignano, Cimino, Villani & Fontana, 2009). In addition, it was observed that the epoxy derivative of elysione, an optical isomer of tridachiapyrone-C (**Atlas 138**), was more abundant in the dark than in the light. It should be a protected form of elysione. In fact, it is stable when exposed to light whereas elysione is rapidly transformed into other isomers including the enantiomer of crispatene (**Atlas 133**). In addition, the acyclic precursor of elysione was detected only from animals maintained in the dark. The general course of events seems to move from propionic acid followed by elongation with seven other propionic units, leading to the acyclic precursor of elysione that is in equilibrium under enzymatic control, with its epoxy derivative. The instability of elysione confirms the "sunscreen" protection after secretion into the mucus.

A population of *Elysia* (=*Tridachia*) *crispata* from Venezuela has been found to contain some polypropionates along with other secondary compounds, including crispatenine (**Atlas 215**), which is a sesquiterpenoid (Gavagnin, Mollo, Castelluccio, Montaro, Ortea & Cimino, 1997; Bourdron, Commeiras, Audran, Vanthuyne, Hubaud & Parrain, 2007). In addition to typical algal metabolites, this species has been found to contain a rather similar sesquiterpenoid, onchidal (**Atlas 216**), which is otherwise known only from the pulmonate *Onchidella* (Gavagnin, Mollo, Montanaro, Ortea & Cimino, 2000). It is remarkable for having adopted all strategies: bioaccumulation, biotransformation, and biosynthesis. It should be mentioned that what has been called *Elysia crispata* from the

Florida Keys turns out to be a distinct species recently named as *Elysia clarki* (Pierce, Curtis, Massey, Bass, Kari & Finney, 2006).

Plakobranchus and *Thuridilla* are the remaining Plakobranchidae. *Plakobranchus* was already mentioned in connection with its use of chloroplasts. In the laboratory it feeds on *Udotea* and *Chlorodesmus* (Jensen, 1992). *Plakobranchus ocellatus* and *Plakobranchus* sp. (**Photo 33**) contain propionate-derived γ-pyrones (**Atlas 142, 143**) that have been thought to be sun screens much like those of *Elysia crispata* (formerly *Tridachia crispata*) and *E. diomedea* (formerly *Tridachiella diomedea*) (Ireland & Scheuer, 1979; Manzo, Ciavatta, Gavagnin, Mollo, Wahidulla & Cimino, 2005). Fu, Hong and Schmitz (2000) also studied a series of polypropionate pyrones from what they identified as *Plakobranchus ocellatus*. Some of these (**Atlas 146-149**) were structurally related to the 9,10-deoxytridachione (**Atlas 126**) that had been found in *Elysia diomedea* whereas (**Atlas 150**) others were structurally related to the tridachiahydropyrone (**Atlas 124**) known from *Elysia crispata* (Gavagnin, Mollo, Cimino & Ortea, 1996). Related polypropionates, ocellapyrones (**Atlas 142-143**) and elysiapyrones (**Atlas 144-145**) were also found, respectively, in *Plakobranchus ocellatus* (Manzo, Ciavatta, Gavagnin, Mollo, Wahidulla & Cimino, 2005) and *Elysia diomedea* (Cueto, D'Croz, Maté, San Martin & Darias, 2005; Díaz-Marrero, Cueto, D'Croz & Darias, 2008).

Thuridilla is mainly tropical. It differs from most other sacoglossans in being, with a few exceptions, brilliantly colored and conspicuous rather than cryptic — a good sign of chemical defense. Furthermore, the more basal members are cryptic, whereas the more derived taxa exhibit aposematic coloration (Gosliner, 1995). The only species of this genus for which data are available is *Thuridilla hopei* (**Photo 34**), which contains three diterpenoids, thuridillin-A, thuridillin-B, and thuridillin-C (**Atlas 378-380**) (Gavagnin, Spinella, Crispino, De Almeida Epifanio, Marin & Cimino, 1993). It feeds upon a very small alga, *Derbesia tenuissima* (Gavagnin, Marin, Mollo, Crispino, Villani & Cimino, 1994). The alga contains a diterpenoid (**Atlas 381**) displaying the very active conjugated dienolacetate moiety. Two thuridillins seem to be oxidized forms of the molecule derived from the food and the other a reduced one. Both mechanisms should protect the mollusk from the toxicity of the algal metabolite. The animal may therefore eat up its local food supply and need protection while moving from one patch of algae to another. At least one other species of this genus, *Thuridilla gracilis* as (*T. ratna*), shown in **Photo 35**, has functional chloroplasts (Wägele & Johnsen, 2001), and that gives the animals one more reason for being out in the sun.

The sacoglossans that have cerata, or are descended from ancestors that did have them, are thought to form a monophyletic unit, and to be the sister group of the Plakobranchidae (Jensen, 1996). A recent molecular study suggests that the "polybranchidae" arose well within the Plakobranchidae (Händler & Wägele, 2007). This alternative would not have much effect upon our general conclusions. The group with cerata is divided into two subunits, the families Stiligeridae and Caliphyllidae.

Among the Stiligeridae, *Costasiella ocellifera* (= *C. lilianae*), shown in **Photo 36**, feeds on the green alga *Avrainvillea longicaulis*. It contains a brominated diphenylmethane derivative, avrainvilleol (**Atlas 170**) that has been shown experimentally to deter feeding by fish quite effectively (Hay, Duffy, Paul, Renaud & Fenical, 1990). Likewise, an as yet unidentified species of *Costasiella* collected in the Gulf of Mannar fed preferentially on *Avrainvillea erecta* even though it had access to several other green algae (Ernesto Mollo, personal communication). *Placida dendritica*, shown in **Photo 37**, feeds upon both *Bryopsis* and *Codium*. According to Trowbridge (1991) it can switch from one of these algal genera to the other, but only with difficulty. The slug makes use of the algal chloroplasts for camouflage, but evidently not as an energy source. However, it contains polypropionate γ-pyrones (**Atlas 100-103**) (Vardaro, Di Marzo & Cimino,

1992). A recent work (Cutignano, Cimino, Villani & Fontana, 2009) suggests that these are synthesized *de novo*, following a biosynthesis (mixed acetate-propionate) similar to that observed in bacteria, but different from that of some fungi, in which there is methylation of polyacetate, and which contain metabolites displaying almost identical structural features. In addition to γ-pyrones *P. dendritica* contains α-pyrones (Placidenes C-F)(**Atlas 104-107**) and a remarkable hydroperoxide (**Atlas 101**) (10-hydroperoxyl placidene A (Cutignano, Fontana, Renzulli & Cimino, 2003).

Ercolania funerea (**Photo 38**), which eats *Chaetomorpha*, contains γ pyrone polypropionates (Vardaro, Di Marzo, Marin & Cimino, 1992). One of these, 7-methyl-cyercene-1 (**Atlas 98**) has also been recovered from media used to culture a phytopathogenic fungus, *Phoma tracheiphila* (Tringali, Parisi, Piattelli & Magnano di San Lio, 1993). Before we jump to the conclusion that some kind of lateral gene transfer or symbiotic organism is responsible for the existence of this metabolite in the mollusks, we should consider a much more straightforward explanation: coincidence. There are a wide variety of cyercenes and it is hardly surprising when one of them happens to have the same structure as a compound from some unrelated organism. Furthermore, as mentioned above, fungal propionic units are biosynthesized from acetate and then methylated, whereas opisthobranchs really do make them from propionate.

Among the Caliphyllidae, endogenous polypropionates (**Atlas 88-91**) are also found in *Cyerce cristallina*, (**Photo 39**) of uncertain feeding relationships (Di Marzo, Vardaro, De Petrocellis, Villani, Minei & Cimino, 1991; Vardaro, Di Marzo, Crispino & Cimino, 1991), and in *Cyerce nigricans* (**Atlas 86-87**) which feeds upon *Chlorodesmis* (Roussis, Pawlik, Hay & Fenical, 1990) and contains the diterpenoid chlorodesmin (**Atlas 383**). *Mourgona germaniae* is known to obtain prenylated bromohydroquinones such as cyclocymopol (**Atlas 169**) from *Cymopolia barbata*, the calcareous green alga upon which it feeds (Högberg, Thompson & King, 1976). This compound is structurally related to the brominated diphenylmethane derivative found in the *Costasiella pcellifera-Avrainvillea longicaulis* pair. It also secretes a viscid mucus that is toxic to various animals (Jensen, 1984). *Caliphylla mediterranea*, shown in **Photo 40**, feeds upon *Bryopsis plumula*, on which it is well camouflaged thanks, in part, to symbiotic chloroplasts. It seems not to have chemical defense (Di Marzo, Marin, Vardaro, De Petrocellis & Cimino, 1993).

Propionates that are synthesized *de novo* are often associated with defensive autotomy of the cerata, and in some cases these compounds have been found to stimulate regeneration of the cerata (Di Marzo, Marin, Vardaro, De Petrocellis, Villani & Cimino, 1993). Among the Stiligeridae, *Ercolania funerea* (**Photo 38**) autotomizes readily (Vardaro, Di Marzo, Marin & Cimino, 1992). Regeneration in this species occurs less rapidly than it does in *Cyerce cristallina*. However, *Placida dendritica*, shown in **Photo 37**, does not autotomize. Its polypropionate γ-pyrones seem to be used only in chemical defense because they do not stimulate regeneration (Di Marzo, Marin, Vardaro, De Petrocellis & Cimino, 1993). Like other γ-pyrones mentioned above, cyercene A (**Atlas 88**) from *Cyerce cristallina* is able to link singlet oxygen at a significantly higher rate than the corresponding α-pyrone isomer. The placidenes and cyercenes of Caliphyllidae are involved in both defense and regeneration. *Cyerce cristallina* autotomizes the cerata when it is attacked. These regenerate rapidly. One of the metabolites, which is found only in the cerata, has been shown to stimulate regeneration in experiments with *Hydra*. This well-known and much-studied freshwater cnidarian is an animal that responds to many growth and regeneration factors (Di Marzo Marin, Vardaro, De Petrocellis & Cimino, 1993). *Caliphylla mediterranea* is highly camouflaged rather than aposematically colored, and does not autotomize readily. Polypropionates have not been detected in this species.

A recent study on *Aplysiopsis formosa* confirmed the presence of pyrones in Stiligeridae. In fact, the mollusk contained aplysiopsenes A-D, which are α-pyrones with a limited extra-ring con-

jugation (**Atlas, 108-111**) (Ciavatta, Manzo, Nuzzo, Villani, Cimino, Cervera, Malaquias & Gavagnin, 2009).

One might wonder if perhaps these *de-novo* synthesized compounds originated as defensive metabolites and then were pressed into service as stimulants to regeneration. On the other hand, somewhat the opposite scenario also comes to mind. If the original function was not defense, but some kind of signaling function within the body of the slugs, then the ancestral metabolite could have been modified so as to function in either defense or wound healing, or perhaps both. Such a scenario is not without precedent among opisthobranchs. Some nudibranchs, as we shall see, have defensive metabolites that are derived from prostaglandins and are likewise implicated in the regeneration of autotomized cerata.

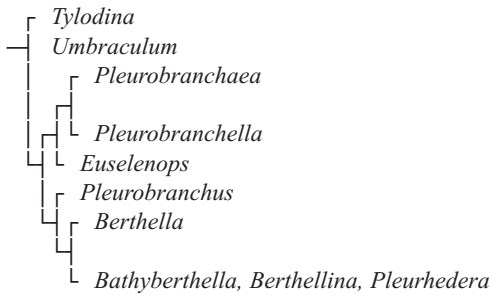
Summing up the historical aspect of this chapter, the sacoglossans seem to have started out as burrowing herbivores that became able to exploit siphonaceous algae as a food source. The defensive use of caulerpenyne and its derivatives from the algae allowed them to move above the substrate. They then diversified, exploiting a variety of different algae as a source of food and defensive metabolites. When the shift meant a loss of protection from metabolites in the food, the animals often switched to *de novo* biosynthesis of polypropionates. The apparent lack of polypropionates in shelled sacoglossans could mean that they were lost in that lineage. But it may mean that they were present at low levels and had some other function, as we have suggested for other groups of opisthobranchs. The involvement of polypropionates in the regeneration of autotomized cerata makes one suspect that their absence may be more apparent than real. Correlated with reliance upon chloroplasts for energy and a habit of basking in the sun is the presence of photoactive polypropionates. These might function as sunscreens, as defensive chemicals, or very likely as both.

Chapter IX

Notaspidea

Notaspidea is a small group of animals that have long been considered the closest relatives of a much larger one, Nudibranchia. As was explained earlier, the two orders have been grouped together in an assemblage called Acoela. Recent molecular research has failed to confirm the monophyly of Notaspidea as traditionally conceived. *Tylodina* and *Umbraculum* are therefore removed and relegated to a kind of taxonomic Limbo, and Notaspidea is restricted to the remainder, which, together with Nudibranchia form a taxon called Nudiplura. Here we will not take a definite stand on this matter but treat the lower "notaspideans" first, with the understanding that they are *incertae sedis* within Opisthobranchia. After all, we have to put them somewhere. Notaspideans (in either the broader or the narrower sense) have many characters that make them seem more primitive than, and transitional to, nudibranchs. As usual, however, they have diverged since common ancestry and they have undergone a minor adaptive radiation of their own. Some authors have speculated that the nudibranchs evolved polyphyletically from more than one group of notaspideans. This notion has not been substantiated and most recent workers do not take it seriously. However the possibility that Notaspidea is paraphyletic remains open.

The following tree indicates probable relationships among the notaspideans that are of interest here:



This tree shows an unresolved trichotomy for *Tylodina*, *Umbraculum* and other notaspideans to reflect the fact that the position of *Tylodina* has been rendered questionable by molecular studies. The families Umbraculidae and Tylodinidae have traditionally been treated as the first branch of the notaspideans (e. g., Vayssière, 1885). Schmeckel (1985) created an order Umbraculomorpha for them. For our purposes we need concern ourselves with just two genera, *Umbraculum* and *Tylodina*. Willan (1987) published a phylogenetic tree in which the families Tylodinidae and Umbraculidae were treated as the sister group of all the rest of the Notaspidea. Cervera, Gosliner, Garcia-Gomez and Ortea (2000) criticized this work on the grounds that it was not based on parsimony. Nonetheless they maintained the traditional view, although most of the characters uniting *Umbraculum* and *Tylodina* may be plesiomorphies. One molecular study shows a close relationship between these two genera but places them next to *Runcina* and Cephalaspidea ss., quite distant from Nudipleura (Grande, Templado, Cervera & Zardoya, 2004). Gosliner (1991) gave good arguments for keeping *Umbraculum* in Notaspidea. Some authors have noted its similarity to anaspideans: Thompson (1973) with respect to spermatozoon ultrastructure and Van den Biggelaar and Haszprunar (1996) with respect to egg cleavage patterns. Be this as it may, animals of both genera have cap-shaped shells, a feature that does not occur in any other opisthobranchs. The shell of *Tylodina* is larger, relative to the rest of the body, than that of *Umbraculum*. In *Umbraculum* it does not fully cover the body, though it seems to have done so in some fossil forms (Valdés & Lozuet, 2000). Animals of both genera feed upon sponges, and this may be a primitive condition for both Notaspidea and Nudibranchia.

Tylodina fungina (**Photo 41**) from the Gulf of California was found to contain metabolites including an ester of a brominated alkaloid derived from unspecified species of sponges of the genus *Verongia*, now synonymized with *Aplysina* (Demospongiae: Verongida: Aplysinidae) (Andersen & Faulkner, 1972). The Mediterranean species *Tylodina perversa* derives similar metabolites from *Aplysina crassa*, formerly called *Verongia aerophoba* (Teeyapant, Kreis, Wray, Witte & Proksch, 1993). The notaspideans are also able to feed upon another closely-related sponge (Thoms, Ebel & Proksch, 2006). The metabolites are brominated alkaloids thought to be derived from 3,5-dibromotyrosine. These metabolites (**579-580**) deter predation on the sponges (Thoms, Wolff, Padmakumar, Ebel & Proksch, 2004). They are sequestered by the slugs and occur at high levels in the mantle, mucus, reproductive organs and egg masses (Ebel, Marin & Proksch, 1999). Some tissues of the sponge contain cyanobacteria, and the slugs feed preferentially on the tissues that contain them (Becerro, Turon, Uriz & Templado, 2003). Uranidine (**Atlas 162**) a phenolic pigment that darkens upon exposure to the air, is also derived from the sponge (Cimino, De Rosa, De Stefano, Spinella & Sodano, 1984; Cimino & Sodano, 1994).

Photo 42 shows a specimen of *Umbraculum mediterraneum* perched atop a sponge. The shell, which is much reduced, can barely be seen, but the gill is quite conspicuous just beneath it. The integument of *Umbraculum mediterraneum* contains fatty acid esters that have been shown to be

toxic to fish (Cimino, Crispino, Spinella & Sodano, 1988; Gavagnin, Spinella, Cimino & Sodano, 1990). Two are diacylglycerols, such as umbraculumin-A (**Atlas 519**) and umbraculumin-B (**Atlas 521**) whereas umbraculumin-B (**Atlas 520**) is a bis hydroxybutyric acid ester (Cimino, Spinella, Scopa & Sodano, 1989). This material occurs in parts of the sponge that are adjacent to the slug, evidently because the metabolites have diffused into it, and not because it is an inducible defense (Cimino & Sodano, 1994). The structure of the diacylglycerol umbraculumin-A has been justified by synthesis (DeMedeiros, Herbert & Taylor, 1990).

In the remaining Notaspidea the shell is much reduced, or even absent (in the adults), and is not cap-shaped. They form a clade with two major branches, according to Willan (1987). The first branch consists of the genera *Euselenops*, *Pleurobranchella*, and *Pleurobranchaea*. The second branch has *Pleurobranchus* as the sister group of *Berthella* + *Bathyberthella*, *Berthellina* and *Pleurhedera*. Basically the same arrangement is given by Cervera, Gosliner, Garcia-Gomez & Ortea (2000).

This group of notaspideans is noteworthy for defensive dermal glands in the skin that produce sulphuric acid (Panceri, 1869; Thompson & Slinn, 1969). One survey found that acid was secreted from the integument upon disturbance by all of the species of notaspideans studied: *Pleurobranchaea californica, Pleurobranchus membranaceus, Pleurobranchus strongi, Berthella plumula*, and *Berthellina citrina* (Gilette, Saeki & Huang, 1991). Thus defensive acid secretion occurs in all of the main branches mentioned above. The presence of acid secretion has perhaps dissuaded chemists from paying much attention to other defensive chemicals, but some very interesting examples have nonetheless been studied. There is also an acid-producing gland that opens into the anterior part of the gut, near the mouth. *Pleurobranchaea californica* uses this acid (pH 1.2) secretion in subduing prey (Morse, 1984).

Pleurobranchaea is thought to be a generalized scavenger, but it also is known to feed upon slow-moving animals (Willan, 1984). It has a distinct preference for sea anemones and other cnidarians, but it has also been found to feed upon sponges, various nematodes, polychaetes, amphipods, opisthobranchs, ophiuroids, squid, and fish parts (Cattaneo-Vietti, Burlando & Senes, 1993). *Pleurobranchaea meckelii*, shown with its eggs in **Photo 45**, has been found to contain labdane aldehydes (**Atlas 351-352**) in the skin (Ciavatta, Villani, Trivellone & Cimino, 1995). No data on chemical defense are available for other notaspideans of the first major branch.

In the second major branch, the skin of *Pleurobranchus membranaceus* (**Photo 44**) has been found to contain polypropionates called membrenones (Ciavatta, Trivellone, Villani & Cimino, 1993). The structure of (-)-membrenone-C (**Atlas 85**) has been confirmed by synthesis (Perkins & Sampson, 2001). The possibility that these are biosynthesized *de novo* seems reasonable, but no experimental work has been done to test that hypothesis. This species, which has a large acid-secreting gland in its buccal cavity, has been observed to feed upon the ascidians *Botryllus schlosseri* and *Ascidia mentula* (Thompson & Slinn, 1959). Both *Pleurobranchus albigutattus* and *P. forskalii* that had been feeding on the tunicate *Lissoclinum* contained the kind of chlorinated diterpenes that occur in such food items (Fu, Palomar, Hong, Schmitz & Valeriote, 2004).

The Mediterranean *Pleurobranchus testudinarius* (**Photo 43**) has two triterpenoids, testudinariol A and B (**Atlas 515-516**), that resemble those of sponges in the skin and mucus (Spinella, Mollo, Trivellone & Cimino, 1997). An unidentified species of *Pleurobranchus* from the South China Sea, already mentioned above, contains the halogenated algal sesquiterpene pacifenol (**Atlas 277**) and also testudinariol B (**Atlas 516**) (Carbone, 2007). Since these animals are carnivorous, it seems likely that the sesquiterpenoid is derived from food, perhaps from herbivorous prey, or perhaps ingested incidentally. On the contrary, the triterpenoids have been found in the mantle, but not in the digestive gland, suggesting *de novo* synthesis. Another study on *Pleurobranchus forskalii*

revealed a cyclic peptide, keenamide-A (**Atlas 638**) (Wesson & Hamann, 1996). This is again one of a class of metabolites that commonly occur in tunicates. It is worth pointing out that tunicates have cells that contain highly acidic secretions. *Berthella* and *Berthellina* on the other hand mainly feed on sponges, especially Demospongiae, though there are reports of their feeding on calcareous sponges and corals (Willan, 1984). Except for the acid secretions noted above, chemical defense has not been recorded from these pleurobranchs. Since they are common animals this probably does not mean that nobody has studied them.

There exists a curious pattern of similarity between the metabolites of some notaspideans and those of intertidal limpets (Díaz-Marrero, Dorta, Cueto, Rovirosa, San-Martín, Loyola & Durias, 2003). *Pleurobranchaea meckelii* has two diterpenes (**Atlas 351, 352**) that resemble one (**Atlas 353**) from a pulmonate, *Trimusculus reticulatus*. *Pleurobranchus membranaceus* has a polypropionate, membrenone-C (**Atlas 85**) that resembles one, vallartanone-D (**Atlas 84**) from another pulmonate limpet, *Siphonaria maura*. *Pleurobranchus testudinarius* has triterpenoids, testudinariols (**Atlas 515-516**) much like limatulone (**Atlas 517)** of a prosobranch limpet, *Lottia limatula*. Although the two pulmonates are closely related, and are distant relatives of opisthobranchs, the prosobranch represents a very early branch of the gastropods, not at all close to either pulmonates or opisthobranchs. The limpets in question share a common habitat and a cap-shaped shell, but there is no evident causal basis for this pattern. So far as we can tell, it is just a curious coincidence.

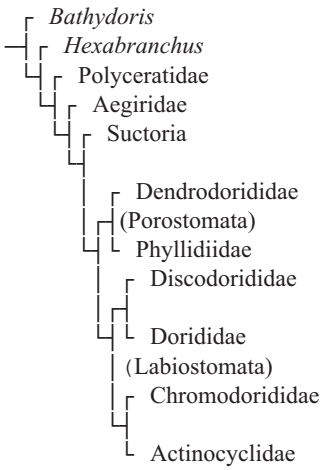
A close relationship between the two major groups of notaspideans and the nudibranchs might be taken as an indication that sponge feeding was the ancestral state in Notaspidea. But these relationships are most uncertain. At any rate, within the second group, dermal acid secretion as a defensive mechanism is generally present and it evidently represents the ancestral state. Acidic secretion from the gut is used in dealing with a variety of prey organisms. Some metabolites derived from food are used defensively. The polypropionates of *Pleurobranchus membranaceus* may be a replacement for dietary metabolites from some ancestral food item, but it is not clear from the data whether this is what happened or what that food might have been.

As to the nudibranchs themselves, they are commonly divided into two main groups: Holohepatica and Cladohepatica. The names refer to the digestive gland or hepatopancreas being entire (holohepatic) or branched (cladohepatic). Alternative names for these two groups are Anthobranchia and Cladobranchia and Euctenidiacea and Actenidiacea. The term Cladobranchia is not very appropriate because it implies that the gills themselves are branched. Holohepatica is equivalent to one of the four traditional orders of nudibranchs, Doridacea, whereas Cladohepatica consists of the other three: Dendronotacea, Arminacea and Aeolidiacea. It is likely that Dendronotacea and Arminacea are paraphyletic grades. The cladohepatic condition is associated with feeding on cnidarians and is obviously derived from the holohepatic condition. The ancestral food of the nudibranchs and their pleurobranch forebears was very likely sponges, but there is no compelling evidence for that.

Chapter X

Nudibranchia: Doridacea

The following tree represents the likely relationships among the major groups of dorid nudibranchs as presently understood. More detailed trees for some of these groups are given below. This one shows the more basal relationships.



Much of the discussion about the origin of the dorid nudibranchs, and of nudibranchs in general, has focused upon the primitive genus *Bathydoris* (Minichev, 1969; Wägele, 1989). The name of this genus is taken from its deep-water habitat, but it ranges into shallower waters in the Antarctic. Evans (1914) considered the single species of *Bathydoris* that he studied to be omnivorous, though the sponges in its gut suggested that it had "predilections" for them. More recently *Bathydoris hodgsoni* has been found to contain hodgsonal (**Atlas 243**), a 2-substituted drimane sesquiterpene having a skeleton that is typical of sponges (Iken, Avila, Ciavatta, Fontana & Cimino, 1998; Avila, Iken, Fontana & Cimino, 2000); the drimane skeleton is also biosynthesized by porostome nudibranchs of the family Dendrodorididae (*q.v.*). That this metabolite is concentrated in the skin makes it seem likely that it is synthesized *de novo*. If it is not synthesized *de novo*, the obvious interpretation is that *Bathydoris* has expanded its diet yet remains dependent on sponges for metabolites derived from food. If, on the other hand, it is synthesized *de novo*, then it would seem that eating sponges was the original condition and that the relative omnivory is secondary.

On the other hand, Valdés (2004) proposes essentially the opposite scenario. He suggests that the ancestral condition was as in *Bathydoris*, with an omnivorous organism having *de novo* synthesis of protective metabolites. The animals then shifted to feeding upon sponges. Subsequently *de novo* synthesis has been replaced by the sequestration of metabolites from food in various groups of dorids. The trouble with this scenario is that dorid nudibranchs of various groups biosynthesize a variety of metabolites *de novo*. They are related to dorids that derive those metabolites, or very similar ones, from their food. The ancestral dorid could not have possessed the capacity for *de novo* biosynthesis in general or in the abstract, but would have had to biosynthesize some particular metabolite. Valdés (2002) also maintains that *Bathydoris* gave rise to two lineages of nudibranchs, one of these being the dorids and the other all the rest of the nudibranchs. Although the treatment of the data would seem to be sound, this inference of paraphyly is based upon the presence of a few characters that may have evolved in parallel.

Hexabranchus sanguineus, commonly known as the "Spanish dancer," is a large, brightly-colored dorid that is quite common in the tropics. As can be seen in **Photo 46**, the animal swims by a dorso-ventral undulatory movement. It shows a "startle response" (Edmunds, 1968). *Hexabranchus* is considered the sister group of all dorids except for *Bathydoris*. It contains macrolides that are evidently derived from sponges of the genus *Halichondria* (Demospongiae: Halichondrida: Halichondriidae) (Kernan, Molinski & Faulkner, 1988; Matsunaga, 2006). The macrolides, such as ulupualide B (**Atlas 667**), which have been shown to deter predators, occur in

the digestive gland, the integument, the reproductive organs and the egg masses (where they were first discovered) (Roesener & Scheuer, 1986; Pawlik, Kernan, Molinski, Harper & Faulkner, 1988; Matsunaga, Fusetani, Hashimoto, Kanahisa, Koshi, Noma, Noguchi & Sankawa, 1989). An esterified carotenoid pigment, hurgadin (**Atlas 513**), is located in portions of the mantle, where it probably functions both as a visual signal and in chemical defense (Guo, Gavagnin, Mollo, Trivellone, Cimino & Fakhr, 1988). This molecule seems to be a protected form of a conjugated dialdehyde, structurally analogous to many other defensive chemicals. These dialdehydes are similar to those found in the porostome family Dendrodoridídae. A single specimen collected in the South China Sea contained three sesquiterpenes and one diterpene (**Atlas 459**) (Zhang, Gavagnin, Guo, Mollo, Ghiselin & Cimino 2007). Two of the sesquiterpenes (**Atlas 315-316**), displaying the same skeleton but with a formamide substituent at different positions, were sequestered in the integument, but the usual macrolides were not detected. There were also isocyanides like those found in the porostome family Phyllidiidae. Isocyanides are also found in *Halichondria* (Demospongiae: Halichondrida: Halichondriidae) (Molinski, Faulkner, Van Duyne & Clardy, 1987). As if that were not enough, there were also 15-20 carbon terpenoids like those found in Chromodorididae. This is a truly remarkable assemblage of metabolites. The diversity of secondary compounds stands in stark contrast to the specialized arsenals of other nudibranchs. This animal is a generalist with respect both to the sponges on which it feeds and the metabolites that it uses defensively.

The remaining dorid nudibranchs have traditionally been divided into Phanerobranchia and Cryptobranchia. The cryptobranchs are able to protect their gills by withdrawing them into a pouch. The gills of phanerobranchs are not entirely unprotected, however. They are retractile and may be surrounded by projections of the body. The ability to withdraw the gills into a pouch is obviously a derived character, and it serves as a good synapomorphy for Cryptobranchia, indicating the monophyly of the group. On the other hand the phanerobranchs have the primitive condition for dorids as a whole so the group may well be a paraphyletic grade. The Phanerobranchia have been divided into Suctoria and Nonsuctoria, based on the presence or absence of a pump used in feeding.

Again, Suctoria can be diagnosed on the basis of a derived character, but Nonsuctoria is very likely a paraphyletic grade, and Suctoria appears to be the sister group of Cryptobranchia (Fahey & Gosliner, 2004). The sequence leading to Cryptobranchia is therefore a series of branches leading to *Bathydoris*, then *Hexabranchus*, then Polyceratidae, then Aegiridae, then Suctoria. Many organisms in the more basal lineages contain alkaloids, often from calcareous sponges or bryozoans upon which the animals feed.

Among the first branch, Polyceratidae, alkaloids have been recorded, derived from bryozoans and other soft bodied animals or sometimes synthesized *de novo*. **Photo 47** shows a specimen of *Polycera elegans* crawling on a bryozoan colony. *Triopha catalinae* and *Polycera tricolor* have diacylguanidine alkaloid, triophamine (**Atlas 581**) (Gustafson & Andersen, 1982). There is good evidence for *de novo* synthesis of this compound (Graziani & Andersen, 1996; Kubanek & Andersen, 1997). The animals were able to incorporate both acetate and butyrate in experiments with the labeled molecules. Limaciamine (**Atlas 582**), another diacylguanidine, occurs in the integument of *Limacia clavigera* (Graziani & Andersen, 1998).

Other nudibranchs of the family Polyceratidae, currently treated as the subfamily Nembrothinae, are remarkable for the manner in which they exploit a series of alkaloids called tambjamines (**Atlas 583-586**) (Carté & Faulkner, 1983, 1986). These compounds are evidently the products of symbiosis of bacteria with bryozoans. *Tambja capensis* (**Photo 48**) contains two tambjamines (**Atlas 583, 587**) and a blue tetrapyrrole pigment, which is a stereoisomer of one found (**Atlas 589**) in some species of *Nembrotha* (Paul, Lindquist & Fenical, 1990) from the blue bry-

ozoan *Bugula dentata* upon which it feeds (Rapson, 2004, cited in Davies-Coleman, 2006). *Tambja verconis* and *Tambja morosa* (**Photo 49**) obtain a series of alkaloids from *Bugula dentata* (Appleton & Copp, 2005). Since they contain a wider variety of metabolites than the bryozoans do, it has been suggested that the slugs biotransform them. *Tambja abdere* and *T. eliora* (**Photo 50**) obtain tambjamines by feeding on the bryozoan *Sessibugula translucens*. These two nudibranchs are eaten by another nudibranch, *Roboastra tigris*, shown in **Photo 51**, which then uses the tambjamines in its own defense. *Roboastra europea*, shown in **Photo 52**, is strikingly aposematic. In its tropical and semi-tropical environments *Roboastra* is often found crawling about quite openly and in broad daylight. Therefore it would seem that the chemical defense is very effective.

Another source of tambjamines (**Atlas, 587, 588**) among nudibranchs of the subfamily Nembrothinae are ascidians of the genus *Apatozoa*, which are fed upon by *Nembrotha* (Paul, Lindquist & Fenical, 1990; Lindquist & Fenical, 1991). Not surprisingly, given their food, a blue tetrapyrrole (**Atlas 589**) has been isolated from *Nembrotha kubaryana*, shown in **Photo 53** (Karuso & Scheuer, 2002).

A recent phylogenetic analysis based on both molecular and morphological evidence has clarified the relationships among the various genera and species within this assemblage (Pola, Cervera & Gosliner, 2007). Both *Nembrotha* and *Roboastra* are holophyletic, whereas *Tambja* is paraphyletic. *Roboastra* falls well within a clade of *Tambja* and some species of *Tambja* are closely related to *Nembrotha*. In other words, *Roboastra* is a modified *Tambja* that has shifted from bryozoans to nudibranchs as a source of food and metabolites, and *Nembrotha* would seem to have had a similar history, but be derived from a different lineage and seems to have shifted from bryozoans to tunicates. The diet of the mollusks here is obviously not dependent upon the taxonomic position of the organisms that are eaten. Rather, in the course of evolution they have shifted from feeding upon one soft-bodied animal to another, all of which contain metabolites that get pressed into service in chemical defense.

In the Aegiridae, the only animals for which data on chemical defense are available are *Aegires gardineri* (**Photo 54**) and *A. citrina* (both as *Notodoris*), which derive 2-iminoimidazole alkaloids such as naamidine-A (**Atlas 590**) from calcareous sponges of the genus *Leucetta* (Calcarea: Calcinea: Clathrinida: Leucettidae) (Carmely, Bowden & Coll, 1993; Carmeley, Ilan & Kashman, 1989; Alvi, Crews & Loughhead, 1991; Alvi, Peters, Hunter & Crews, 1993; Carroll, Bowden & Coll 1993). (The genus *Notodoris* has been synonymized with *Aegires* by Fahey & Gosliner, 2004).

In Suctoria of the family Onchidoridídae, there are also some animals that contain terpenoids, supposedly derived from the bryozoans on which these animals feed. *Adalaria loveni* contains a degraded triterpenoid, lovenone (**Atlas 518**) (Graziani, Allen & Andersen, 1995). In *Acanthodoris nanaimoensis*, two specimens of which are shown in **Photo 55**, there are sesquiterpenoids such as nanaimoal (**Atlas 227**), acanthodoral (**Atlas 229**), and isoacanthodoral (**Atlas 228**) (Ayer, Andersen, He & Clardy, 1984; Ayer, Hellou, Tischler & Andersen, 1984; Liu, Ulibarri & Nelson, 1990). This group of compounds was shown to be biosynthesized *de novo* (Graziani & Andersen, 1996); the animals incorporated labeled acetate. *Acanthodoris hudsoni*, a less common species that occurs in the same area, contains the same metabolites, but in a different ratio: 1:10:40 instead of 40:10:1 (Andersen, Desjardine & Woods, 2006). Animals of both species have a pleasant smell.

The remaining dorids are cryptobranchs. Typically they feed upon sponges of the class Demospongiae. Cryptobranchia as here conceived includes the Porostomata, or radula-less dorids, in some of which the retractile, flower-like gill has been lost. Valdés (2002), whose phylogenetic arrangement we will follow here, treats the Porostomata as the sister group of all other Cryptobranchia, for which he has introduced the name Labiostomata.

Porostomata is now understood, on the basis of both molecular and anatomical evidence, to be

a monophyletic group (Valdés & Gosliner, 1999; Valdés, 2003). Its polyphyly was suspected on the basis of the ease with which the radula might be lost and the many differences between the two main groups (Brunckhorst, 1993). The radula, however, has not simply been lost. The pharynx has been converted into a long, tubular structure that allows the animals to feed suctorially on the tissues of sponges. Porostomata is now divided into three families, Maneliidae, Dendrodorididae, and Phyllidiidae. In the Mandeliidae and Dendrodorididae the typical dorid gill is retained, whereas in the Phyllidiidae it has been lost and, with few exceptions, replaced by a series of lamellae on each side of the foot. Animals of both of the latter two families have been extensively studied from the point of view of chemical defense.

In the Dendrodorididae two genera are currently recognized: *Doriopsilla* and *Dendrodoris*. In their gonads all of these nudibranchs studied thus far contain drimane sesquiterpenoid fatty acid esters (**Atlas 236**), which may be biosynthesized *de novo*, and in some cases rigorous experimental evidence of this is available. These compounds are transferred to the eggs. They can be broken down into two parts, one of which provides the developing larva with an energy source like ordinary yolk, while the other serves a protective function. In other parts of the bodies of the adults there are sesquiterpenoids (**Atlas 237-239**) that at least sometimes are biosynthesized *de novo*. One of them, olepupuane (**Atlas 237**), delivers poygodial (**Atlas 235**) that is very harmful to predators. The compounds recorded from the nudibranchs are similar, and sometimes identical, to those that occur in sponges. However, there is no evidence for the incorporation of defensive metabolites from the sponges. Evidently these mollusks are descended from ancestors that obtained defensive metabolites from the sponges upon which they fed but they no longer do so, and have not done so since common ancestry of the two genera. Let us now consider the comparative and experimental work on which this inference is based.

Doriopsilla areolata, shown with its coiled egg mass in **Photo 56**, was not found to contain sponge metabolites in the digestive gland (Spinella, Alvarez, Avila & Cimino, 1994; Gavagnin, Mollo, Calado, Fahey, Ghiselin, Ortea & Cimino, 2001). However there are metabolites of that sort elsewhere in the body, suggesting that the animals have evolved the capacity for biosynthesizing them *de novo*. In the gonad, there is a mixture of drimane fatty acid esters (**Atlas 236**) that gets incorporated in the eggs and serves the developing embryo as both a store of energy and a means of defense. Related drimane sesquiterpenoids (**Atlas 237-239, 342**) were detected on the mantle. The sesquiterpenoid (-)*ent*-pallescenscin-A (**Atlas 339**) occurs in the gill, and two of its apparent derivatives in the border of the mantle: 15-acetoxy-*ent*-pallescensin-A (**Atlas 340**) and 2,15-diacetoxy-*ent*-pallescensin-A (**Atlas 341**). An enantiomer of the latter, (+)pallescenscin-A, occurs in the sponge *Dysidea pallescens* (Demospongiae: Dictyoceratida: Dysideidae)(Cimino, De Stefano, Guerriero & Minale, 1975). Surprisingly, the A/B ring junctions of the two series, drimane and pallescenscin, of sesquiterpenoids were the opposite. The observation that in another sponge of the genus *Dysidea* the sesquiterpenoids *ent*-pallescenscin-A and euryfuran with an opposite A/B ring junction were found suggests that the compounds in the nudibranchs might indeed be of dietary origin (Butler & Capon, 1993). However, experiments with labeled mevalonate showed a substantial amount of incorporation in both drimane and pallescensin sesquiterpenoids, clearly indicating *de novo* biosynthesis (Gavagnin, Mollo, Castellucio, Ghiselin, Calado & Cimino, 2001; Gavagnin, Mollo, Castelluccio, Ghiselin, Calado & Cimino, 2001). Experiments with labeled glucose confirmed the mevalonate pathway (Fontana, Tramice, Cutignano, d'Ippolito, Gavagnin & Cimino, 2003). Both the drimane esters and the *ent*-acetoxypallescensin can be derived from a single pool of *trans, trans*-farnesyl diphosphate that isomerizes before cyclization.

Another species, *Doriopsilla pelseneeri*, shown in **Photo 57**, has given rather similar results (Gaspar, Gavagnin, Calado, Castelluccio, Mollo & Cimino, 2005). It was also found to contain, in

addition to drimane sesquiterpenoids, clearly biosynthesized by the mollusks (Cutignano et al., in Press), two furanosesquiterpene alcohols, pelseneeriol-1 (**Atlas 317**) and pelseneeriol-2 (**Atlas 318**), which differ only in relative stereochemistry (being epimers at C-3) as proved experimentally by Gaspar, Cutignano, Ferreira, Calado, Cimino and Fontana (2008). Pelseneeriols are monocyclic furanoterpenes structurally related to microcionins, which are sesquiterpenoids possessed by the Mediterranean sponge *Fasciospongia cavernosa* (Demospongiae: Dictyoceratida: Thorectidae; misidentified as *Microciona toxystila*) (Cimino, De Stefano, Guerriero & Minale, 1975).

Doriopsilla pharpa is protected from crabs and fish by polygodial (**Atlas 235**). (Long & Hay, 2006). Polygodial is a metabolite present in all of the porostome nudibranchs belonging to the genera *Dendrodoris* and *Doriopsilla* that have been studied. Experiments with fish of two species, *Fundulus heteroclitus* and *Chasmodes bosquianus*, showed that the fish learned to avoid pieces of squid containing the metabolite, but that both the mechanisms and the outcomes were different. *C. bosquianus* regurgitated the treated food, and then came to avoid such food irrespective of whether it was treated or not. *F. heteroclitus* did not regurgitate, and learned to avoid only the food that had been treated. The latter mechanism would exclude fewer food items from the diet of the fish and should have various other ecological consequences.

More data, giving patterns similar, but not identical, to those of *Doriopsilla*, are available for the genus *Dendrodoris*. In *Dendrodoris krebsi*, variabilin (**Atlas 472**), a sponge-derived sesterterpene, occurs in the digestive gland but not elsewhere in the body (Gavagnin, Mollo, Calado, Fahey, Ghiselin, Ortea & Cimino, 2001). The gonad, like that of *Doriopsilla areolata*, contains a mixture of drimane fatty acid esters. Again, in the gills there is a metabolite that occurs in sponges, but in this case it is the (+)pallescensin A (**Atlas 344**) enantiomer, not (-)pallescensin A (**Atlas 339**) (which occurs in *D. areolata*). A derivative of it occurs along the rim of the mantle. According to Okuda, Scheuer, Hochlowski, Walker and Faulkner (1983), polygodial (**Atlas 235**) and olepupuane (**Atlas 237**) occur in *Dendrodoris tuberculosa* and *Dendrodoris nigra*, as well as in *Dendrodoris krebsi*. *Dendrodoris denisoni*, from New Zealand, contains olepupuane (**Atlas 237**) and polygodial, as well as a similar compound, cinnamolide (**Atlas 240**), otherwise known from terrestrial plants (Grkovic, Appleton & Copp, 2005). Olepupuane was found to be transformed into polygodial via an intermediate methoxy acetal. However, the origin of polygodial from olepupuane was previously rigorously proved.

In fact, olepupuane is the main metabolite present in the skin of *Dendrodoris limbata* (**Photo 58**). It is a protected form of polygodial that is immediately delivered when the mollusk is molested (Cimino, Sodano & Spinella, 1998). Likewise the gonad of *Dendrodoris limbata* contains a mixture of 7-deacetoxy-olepupuane fatty acids (**Atlas 236**) that provision the eggs and protect them (Cimino, Sodano & Spinella, 1988; Avila, Cimino, Crispino & Spinella, 1991). The *de novo* synthesis of 7-deacetoxyolepupuane (**Atlas 239**) and its derivatives has been established by the incorporation of labeled mevalonic acid (Fontana, Ciavatta, Miyamoto, Spinella & Cimino, 1999). The biosynthetic pathway has been more rigorously confirmed by means of glucose labeled with ^{13}C (Fontana, Villani & Cimino, 2000). 7-deacetoxyolepupuane is the only one of these sesquiterpenoids that is present in the juveniles (Avila, 1993). In the adults it is concentrated in the gills. It plays a pivotal role in the biosynthesis of the defensive compounds. On the one hand it forms drimane esters. It also gives rise to olepupuane, which is found in the mantle and is transformed into the more toxic polygodial (**Atlas 235**), which occurs in the mucus. It also gives rise to 6-acetoxy-olepupuane (**Atlas 238**), which occurs in the mantle, and to 6-β-acetoxypolygodial (**Atlas 241**), which occurs in the mucus. 7-Deacetoxyolepupuane (**Atlas 239**) has also been found in a sponge of the genus *Dysidea* (Demospongiae: Dictyoceratida: Dysideidae), and this led to the suggestion, which we now believe is incorrect, that the metabolites found in the mollusks are of dietary origin

(Garson, Dexter, Lambert & Liokas, 1992).

Dendrodoris grandiflora is closely related to *D. limbata* (Valdés & Gosliner, 1999). It also has the same pattern of drimane sesquiterpenoids. *De novo* synthesis has likewise been established experimentally (Cimino, De Rosa, De Stefano, Morrone & Sodano, 1985; Cimino & Sodano, 1994; Avila, Cimino, Crispino & Spinella, 1991; Fontana, Villani & Cimino, 2000). The steroids of this species have also been studied (Cimino, De Stefano, De Rosa, Sodano & Villani, 1980). Although the digestive gland contains an extraordinary series of typical sponge metabolites, none of these occur elsewhere in the body where they might be used defensively. In the field, *D. grandiflora* seems not to be associated with sponges, suggesting that it feeds, unlike the stenophagous *D. limbata*, upon a wide variety of them, perhaps small ones. In this respect it would be rather like the sacoglossan *Thuridilla* (see above).

Dendrodoris arborescens, from the Pacific, was studied along with *Dendrodoris limbata* and *Dendrodoris grandiflora* in the biosynthetic work of Fontana, Ciavatta, Miyamoto, Spinella and Cimino (1999). The results were essentially the same. Sixteen drimane sesquiterpenoids (**Atlas 252-265**), of which fourteen were new, have been described from a single 2 kg specimen of *D. carbunculosa* (Sakio, Hirano, Hayashi, Komiyama & Ishibashi, 2001).

The other family of Porostomata, Phyllidiidae, consists of several genera, but only four of these have been studied from the point of view of chemical defense: *Phyllidiopsis*, *Phyllidiella*, *Phyllidia* and *Reticulidia*. The first of these genera is thought to have branched off first (Brunckhorst, 1993; Valdés & Gosliner, 1999). These animals have lost the flower-like gill that is present in most dorids. In all but one genus it has been replaced by secondary folds at the side of the foot. So far as is known, all of the animals in this group are defended by isocyanide terpenoid metabolites (**Atlas 283-306**) that occur in the sponges on which they feed. Such isocyanide terpenoids have been recorded from a large number of sponges of the orders Axinellida and Halichondrida. Their presence has been treated as an indication that the two orders are sister groups (Van Soest, 1991). *De novo* synthesis of defensive metabolites has not been recorded for this group of nudibranchs. There is weak but legitimate negative evidence against it, in that animals that are kept isolated do not replenish their defensive metabolites (Chang & Scheuer, 1990).

Phyllidiopsis krempfi has been found to contain the same isocyanide terpenoids (**Atlas 299-302**) as a sponge, *Acanthella cavernosa* (Demospongiae: Halichondrida: Dictyonellida), upon which it feeds (Fusetani, Hirota, Okino, Tomono & Yoshimura (1996). The same sponge is the source for metabolites in *Phyllidia* species noted below. Valdés (2001) observes that *Phyllidiopsis* includes a very large proportion of the Phyllidiidae of deeper waters. Some of these are associated with hexactinellid sponges. *Phyllidiopsis krempfi* lives in shallow water.

Dumdei, Flowers, Garson, and Moore (1997) gave labeled cyanide and thyocyanide to both *Phyllidiella pustulosa* (**Photo 65**) and the sponge upon which it feeds, *Acanthella cavernosa* (Demospongiae: Halichondrida: Dictyonellidae). These precursors gave rise to axisonitrile-2 (**Atlas 287**) in the sponge, and the negative results on the nudibranch are in line with other failures to get evidence for biosynthesis by nudibranchs of this family. In the same species, which they called *Phyllidia pustulosa*, Kassühlke, Potts and Faulkner (1991) found nitrogenous sesquiterpenes (**Atlas 290-294**) such as 4α-isocyanogorgon-11-ene derived from *Halichondria* (Demospongiae: Halichondrida: Halichondriidae). On the other hand, Wright (2003) found that the same species, again referred to *Phyllidia pustulosa*, feeds largely on *Phakellia carduus* (Demospongiae, Halichondrida: Axinellidae). He isolated a new metabolite, 10-isothiocyano-4-cadinene (**Atlas 300**), from them as well as axisonitrile-3 (**Atlas 298**) and various similar sesquiterpenoids. Quite a number of isocyanide terpenoids have been described from this species (see Okino, Yoshimura & Hirota 1996; Fusetani, Hiroto, Okino, Tomono & Yoshimura, 1996; Hirota, Okino, Yoshimura &

Fusetani, 1998). A series of nitrogenous diterpenes (**Atlas 454-457**) and sesquiterpenes (**Atlas 303-305**) were detected in *Phyllidiella pustulosa* from the South China Sea (Manzo, Ciavatta, Gavagnin, Guo & Cimino, 2004). The diterpenes are unusual for *Phyllidia*; all the others studied thus far are sesquiterpenes. Although these metabolites were concentrated in the mantle, they were also found in the internal organs. The main metabolite is an enantiomer of stylotellin, a sesquiterpene that has been found in a sponge identified as belonging to the genus *Stylotella*, which has been synonymized with *Hymeniacidon* (Demospongiae: Halichondrida: Halichondridae).

In *Phyllidia bourguini*, Fusetani, Wolstenholme and Matsunaga (1990) found 9-isocyanopupukeanone (**Atlas 283**) and its epimer, 9-*epi*-9-isocyanopupukeanone (**Atlas 284**).

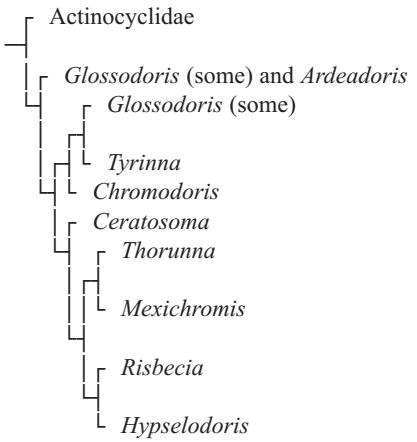
In *Phyllidia flava***,** recorded as *Phyllidia pulitzeri*, Cimino, De Rosa, De Stefano and Sodano (1982) described an isocyanoterpene (**Atlas 298**) obtained from *Axinella* (Demospongiae, Halichondrida: Axinellidae). Yasman, Edrada, Wray and Proksch (2003) described some new 9-thiocyanopupukeanane sesquiterpenes (**Atlas 313, 314**) from *Phyllidia varicosa* and the sponge *Axinyssa aculeata*, upon which it feeds. In the closely related *Phyllidia ocellata* (**Photo 64**) (as *P. pustulosa*) Fusetani, Wolstenholme, Matsunaga, and Hirota (1991) found two new sesquiterpene isonitriles (**Atlas 297, 298**), and some nitrogenous sesquiterpenoids that were previously known from *Acanthella* (**Atlas 295, 296**) (Demospongiae: Halichondrida: Dictyonellidae) (Fusetani, Wolstenholme, Shinoda, Asai, Matsunaga, Onuki & Hirota, 1992). Fusetani, Hirota, Okino, Tomono, and Yoshimura (1996) got a variety of isocyanide and related sesquiterpenes from the sponge *Acanthella cavernosa* (Demospongiae: Halichondrida: Dictyonellidae) and from *Phyllidia ocellata, Phyllidia varicosa*, and *Phyllidiopsis krempfi*. From *Phyllidia varricosa* and the sponge upon which it was found feeding, *Hymeniacidon* (Demospongiae: Halichondrida: Halichondridae), Burreson, Scheuer, Finer, and Clardy (1975) isolated for the first time two tricyclic sesquiterpene isocyanides (**Atlas, 283, 284**). Okino, Yoshimura, Hirota, and Fusetani (1996) reported additional sesquiterpenes from *Phyllidia ocellata* (**Photo 64**)*, Phyllidia varicosa* (**Photo 61**) and *Phyllidella pustulosa*. In *Reticulidia fungia* (**Photo 66**), Tanaka and Higa (1999) found sesquiterpenoids that have a rare functional group, $N{=}CCl_2$ (carbonimidic dichlorides).

The Porostomata thus show two different patterns of metabolite usage. With the exception of the stenophagous *Dendrodoris limbata*, nudibranchs of the family Dendrodorididae feed on a wide variety of sponges, and this euryphagy seems to be facilitated by the capacity for *de novo* biosynthesis. Phyllidiidae evidently are restricted to a group of sponges that contain isocyanide terpenoids. They do not seem to be highly specialized so as to feed only upon a few sponges or utilize only a few metabolites, but this may be just an impression. They utilize very effective defensive metabolites from their food. There would seem to be no advantage for them to engage in *de novo* biosynthesis and that may be the reason why no evidence of it has been found.

Having completed our discussion of the Porostomata, we may now turn to the Labiostomata, which are the remaining dorid nudibranchs. The first lineage, and sister group to all the remainder, consists of the Actinocyclidae, known only from our own unpublished work, and the Chromodorididae, which have been extensively studied and with very interesting results. Here we will present the materials following the tree published by Gosliner and Johnson (1999) and updated through personal communications with Johnson, but will simplify it by not bothering to discuss genera for which no chemical data are available.

This tree differs in some respects from the more subjective one published by Rudman (1984). Rudman treated the genus *Cadlina* as the first branch of the Chromodorididae. According to Johnson it is not a chromodorid, but is the sister group to Aldisidae. We will consider *Cadlina* first without placing it within the tree. According to the scenario of Rudman and Bergquist (2007) the Chromodorididae have specialized so as to feed upon sponges that are not defended by spicules.

The Actinocyclidae they interpret as sometimes feeding on sponges of the family Halisarcidae (Demospongiae: Halisarcida), in which defensive metabolites are unknown, including *Halisarca*, which is neither spiculate nor fibrous. A more basal position of *Cadlina* would make this scenario even more plausible.



The tree shows some species of *Glossodoris* forming a branch together with *Ardeadoris*, and others together with *Chromodoris* and *Tyrinna*. In other words, *Glossodoris* is biphyletic.

Cadlina is a rather flattish animal with protective spicules in its tissues, supplementing its well-developed chemical defense. The main object of research has been *Cadlina luteomarginata*, which is quite common along the more northerly Pacific coasts of North America. There are some differences between the northern and southern populations, and they may actually be two distinct species. Be this as it may, the animal feeds upon a wide range of sponges, and a large number of metabolites derived from sponges have been isolated from it, all of them terpenoids (Gustafson, Andersen, He & Clardy, 1985; Tishler, Andersen, Choudhary & Clardy, 1991). These include isocyanides (Thompson, Walker, Wratten & Faulkner, 1982; Burgoyne, Dumdei & Andersen, 1993). Glaciolide (**Atlas 386**) is a degraded diterpenoid that also occurs in the sponge *Aplysilla glacialis* (Demospongiae: Dendroceratida: Darwinellidae) (Tischler & Andersen, 1989). Other terpenoid metabolites are biosynthesized *de novo* (Dumdei, Kubanek, Coleman, Pika, Andersen, Steiner & Clardy, 1997; Kubanek, Graziani & Andersen, 1997). These are the sesquiterpenoid albicanyl acetate (**Atlas 246**), and the degraded sesterterpenoids cadlinaldehyde (**Atlas 479**) and luteone (**Atlas 480**). Unlike the metabolites derived from food, they do not vary geographically. Experiments using labeled acetate and mevalonate gave good incorporation in all three of these metabolites. It was rigorously demonstrated that both the cadlinaldehyde and the luteone are produced through degradation of a common precursor, which is cleaved in two different places and then gets rearranged. Albicanyl acetate, the most repugnant compound, is accumulated in the eggs as well as the skin. 1α,2α-diacetoxy-albicanyl acetate (**Atlas 244**) is accumulated only in the eggs. The amount of material biosynthesized increases when the eggs are being produced. The other two compounds are mainly found in the integument. The animal has a fruity odor, which has been attributed to the luteone (Hellou, Andersen, Rafii, Arnold & Clardy, 1981; Hello, Anderson & Thompson, 1982).

Cadlina limbaughorum and *Cadlina flavomaculata* selectively accumulate only one or two metabolites from a range of them (Thompson, Walker, Wratten & Faulkner, 1982). These include isonitriles identical to those from the sponge *Axinella* (Demospongiae, Halichondrida:

Axinellidae). The metabolites of two species from the Cantabrian Sea, *Cadlina laevis* and *Cadlina pellucida*, which are closely related to each other, have also been surveyed (Fontana, Gavagnin, Mollo, Trivellone, Ortea & Cimino 1995). A specimen of *Cadlina pellucida* is shown in **Photo 67**. The animals feed upon a wide variety of sponges, making it evident that euryphagy in the genus is a general rule. They contain a mixture of sesquiterpenes (**Atlas 345-349**) and sesterterpenoids (**Atlas 465-466, 481**). The sponges upon which *Cadlina* feeds are defended by spicules, unlike those that are fed upon by the nudibranchs that definitely belong to the Chromodorididae.

As suggested above, *Glossodoris* is probably biphyletic, with one lineage branching off early and another more closely related to *Chromodoris* and *Tyrinna*. We will treat both groups of *Glossodoris* together, followed by *Tyrinna*.

The sample of *Glossodoris* for which data on secondary metabolites are available contains a good representation of the various color groups of Rudman (1985) and therefore probably gives a fair idea of the range of variation. These groups are: 1) The *Glossodoris atromarginata* group; 2) the *Glossodoris pallida* group; 3) the *Glossodoris sedna* group; 4) the *Glossodoris cincta/hikuerensis* group. These assemblages appear to be consistent with the phylogeny worked out by Johnson (in progress). According to Rudman and Bergquist (2007), all of the species of *Glossodoris* for which data are available feed upon sponges of the family Thorectidae. Some of the organisms that have been studied, however, have been found to contain metabolites that are characteristic of Spongiidae and lack the ones that are characteristic of Thorectidae. To make sense out of the data we need to provide some basic information about the metabolites in question.

All of the metabolites here considered are terpenoids. The ones that seem to derive from sponges of the family Spongiidae are typical diterpenoids (Somerville, Mollo, Cimino, Ruangprom & Garson, 2006). Those from Thorectidae are scalarane sesterterpenoids. These include scalaradial itself (**Atlas 486**) and two similar compounds, deacetylscalaradial (**Atlas 487**), and deoxoscalarin (**Atlas 483**). Scalaradial and deacetylscalaradial retain the dialdehyde configuration, whereas deoxyscalarin is a protected form of scalaradial and very likely has been modified by the nudibranchs. There also is a series of scalarane sesterterpenoids that have been modified by the nudibranchs in a manner without precedent in any other organisms (Manzo, Gavagnin, Somerville, Mao, Ciavatta, Mollo, Schupp, Garson, Guo & Cimino, 2007). The animals are able both to reduce the aldehyde group, generally at position 17, and to oxidize the hydroxy substituent at carbon 12. Consequently, whereas there is an acetyl group at position 12 in scalaradial and deoxoscalarin, it has been replaced by an oxo (=O) group in 12-keto scalaranes, such as 12-deacetoxy-12-oxoscalaradial (**Atlas 491**), 12,16,-deacetoxy-12-oxo-scalarafuran (**Atlas 492**), and 19-acetyl-12-deacetoxy-12-oxo-deoxoscalarin (**Atlas 493**). These compounds occur only in the nudibranchs, and they are considered enzymatically transformed detoxification products (Rogers & Paul, 1991; Avila & Paul, 1997; Fontana, Mollo, Ortea, Gavagnin & Cimino, 2000; Gavagnin, Mollo, Docimo, Guo & Cimino, 2004; Manzo, Gavagnin, Somerville, Mao, Ciavatta, Mollo, Schupp, Garson, Guo & Cimino, 2007).

Glossodoris cincta (**Photo 68**) collected from Hurgada on the Red Sea and earlier misidentified as *G. atromarginata*, was found to contain nine spongian diterpenes (**Atlas 429-437**) (Fontana, Mollo, Ricciardi, Fakhr & Cimino, 1997; Wahidullah, Guo, Fakhr & Mollo, 2006). Specimens of *G. cincta* from Guam were found to contain scalaradial and a few other scalarane sesterterpenes including a protected form of scalaradial, 12-deacetyl-12-*epi*-scalaradial, heteronemin (**Atlas 488**), derived from sponges of the genus *Hyrtios* (Demospongiae: Dictyoceratida: Thorectidae) (Rogers & Paul, 1991). The heteronemin occurs in both the body of the slug and the eggs. However, 12-ketoscalaranes have not been reported from this population. Like some of the other sponges upon which the nudibranch has been said to feed, *Hyrtios* belongs to the family Thorectidae, not

Spongiidae (Rudman & Bergquist, 2007). This finding has been reconfirmed.

Specimens of *Glossodoris hikuerensis* from Guam contained scalarane compounds of dietary origin, in this case derived from sponges of the genus *Hyrtios* (Demospongiae: Dictyoceratida: Thorectidae). These did not include scalaradial (**Atlas 486**), 12-deacetoxy-12-oxo-scalaradial (**Atlas 491**), 12,16-deacetoxy-12-oxo-scalarafuran (**Atlas 492**), or any 12-ketoscalaranes, but they did contain heteronemin (**Atlas 488**), in the body but not in the eggs (Rogers & Paul, 1991; Manzo, Gavagnin, Somerville, Mao, Ciavatta, Mollo, Schupp, Garson, Guo & Cimino, 2007). The specimens of *Glossodoris hikeuerensis* from Guam, therefore, have a pattern of metabolites very close to that of *G. cincta* from the same area. Rudman and Bergquist (2006) point out that these species belong to a distinct group within the family, one in which the mechanism of delivering the metabolites is rather different from the rest.

Glossodoris atromarginata presents a rather confusing pattern of geographical distribution and taxonomic uncertainty. At least some of the sponges purportedly of the family Spongiidae upon which they feed actually belong to the family Thorectidae, not Spongiidae (Rudman & Bergquist, 2007). And, indeed, animals of this species collected in India contained three scalarane sesterterpenes including one 12-keto scalarane (Fontana, Cavaliere, Ungur, D'Souza, Parameswaran & Cimino, 1999; Fontana, Ciavatta, D'Souza, Mollo, Naik, Parameswaran, Wahidulla & Cimino, 2001); all these are known from that family of sponges but only one was found to be present in the sponge upon which these nudibranchs fed. Animals from Sri Lanka (referred to as *Casella atromarginata*) on the other hand contained furanoditerpenes typical of Spongiidae (De Silva & Scheuer, 1982). A population from South-East Queensland, Australia, was found to contain a series of furanoditerpene compounds of the sort that occur in Spongiidae from a sponge that has been identified as *Spongia* sp. (Demospongiae: Dictyoceratida: Spongiidae) (Somerville, Mollo, Cimino, Rungprom & Garson, 2006). One of these (spongia-13(16),14-dien-3-one) (**Atlas 438**) was recovered from the internal organs as well as the mantle, three only from the internal organs, two from the mantle and one from the mantle glands. Surprisingly, the less polar compound (**Atlas, 433**) was detected only in some mantle glands.

More accurate studies on *G. atromarginata* from China confirmed the ability of the mollusk to selectively accumulate the fully acetylated spongiane in the mantle glands (E. Mollo, unpublished data) whereas the corresponding alcohol was detected mainly in the digestive glands. The acetate was extremely active as a feeding deterrent against the shrimp *Palaemon elegans*. Like the Sri-Lankan animals these ones depart from the usual pattern in *Glossodoris* of feeding only on sponges of the family Thorectidae rather than Spongiidae. There seems to have been a shift within *Glossodoris* from one taxon of sponges to another. The possibility that this is more than one species of nudibranch has not been excluded.

Glossodoris sedna (as *Chromodoris*) was found to contain some homoscalarane derivatives (**Atlas 509-512**) with additional carbon atoms (Hochlowski, Faulkner, Bass & Clardy, 1983). As these tetracyclic terpenes were variable it was inferred that they were of dietary origin. Specimens of *Glossodoris sedna* from Costa Rica likewise contained only homoscalarane compounds of dietary origin (Fontana, Mollo, Ortea, Gavagnin & Cimino, 2000).

Specimens of *Glossodoris dalli* from Costa Rica likewise contained scalarane compounds of dietary origin (Fontana, Mollo, Ortea, Gavagnin & Cimino, 2000). These included the modified form deoxoscalarin (**Atlas 483**) but no 12-ketoscalaranes (Manzo, Gavagnin, Somerville, Mao, Ciavatta, Mollo, Schupp, Garson, Guo & Cimino, 2007).

Glossodoris pallida (**Photo 69**) from Guam has been found to contain scalaradial (**Atlas 486**) (as the main metabolite), deacetylscalaradial (**Atlas 487**), and deoxoscalarin (**Atlas 483**) (in both the body and the eggs), but no 12-ketoscalaranes (Rogers & Paul, 1991; Manzo, Gavagnin,

Somerville, Mao, Ciavatta, Mollo, Schupp, Garson, Guo & Cimino, 2007). It is said to feed almost exclusively on sponges of the genus *Cacospongia* (Demospongiae: Dictyoceratida: Thorectidae), for which there have been some problems of identification. According to Rudman and Bergquist (2006) it feeds on *Semitaspongia* (Demospongiae: Dictyoceratida: Thorectidae).

On the other hand, *Glossodoris pallida* from Hainan contains deoxoscalarin, but neither deacetylscalaradial nor scalaradial itself. It does contain six other scalarane compounds (**Atlas 491-496**), including four 12-ketoscalaranes among which is 12-deacetoxy-12-oxoscalaradial (**Atlas 491**) (Manzo, Gavagnin, Somerville, Mao, Ciavatta, Mollo, Schupp, Garson, Guo & Cimino, 2007).

A very similar distribution pattern has been found in *Glossodoris rufomarginata* (**Photo 70**) from Hainan (Gavagnin, Mollo, Docimo, Guo & Cimino, 2004), but fewer metabolites were recorded. Again, it contains deoxoscalarin and some 12-ketoscalaranes, including 12-deacetoxy-12-oxoscalaradial (**Atlas 491**), but only three of them, all of which occur in *G. pallida*. *G. rufomarginata* was misidentified as *Chromodoris youngbleuthi* and said to feed upon a spongid, whereas it actually feeds upon a thorectid (Terem & Scheuer, 1986; see Rudman & Bergquist, 2007).

Glossodoris averni (**Photo 71**), from Mooloolooba, Australia, again displayed a rather similar pattern (Manzo, Gavagnin, Somerville, Mao, Ciavatta, Mollo, Schupp, Garson, Guo & Cimino, 2007). Again, it contains only one scalaradial epimer, deoxoscalarin (**Atlas 481**), and some 12-ketoscalaranes, including 12-deacetoxy-12-oxoscalaradial (**Atlas 491**), but only two of them plus one other.

Glossodoris vespa (**Photo 72**), also from Mooloolooba, Australia, contained no scalaradial epimers, but it did contain one 12-ketoscalarane, namely12-deacetoxy-12-oxo-scalaradial (**Atlas 491**), and also another scalarane derivative, 16-deacetoxyscalarafuran (**Atlas 498**).

Very little is known about the chemical defense of *Tyrinna*. It has reduced spicules, hinting at a trend that goes on within the family. *Tyrinna nobilis* from Patagonia was found to contain a mixture of furanosesquiterpenoids (**Atlas 326**) and a seco-11,12-spongian diterpenoid, tyrinnal (**Atlas 449**) (Fontana, Muniaín & Cimino, 1998).

Chromodoris is a large and mainly tropical to semitropical genus that has been extensively sampled by natural products chemists. As a general rule, they contain rearranged spongiane diterpenoids that are concentrated in the mantle rim, rather than in mantle dermal formations. They tend to be quite colorful, as the name suggests. A particularly striking example is *Chromodoris quadricolor*, shown in **Photo 73**. Like *Cadlina* they are generally rather stout and flattish animals, but unlike *Cadlina* they lack spicules in the integument. The phylogeny of the genus has been studied, but not to the extent that we would like for our purposes. A recent molecular study indicates that the group can be divided into two clades that can be diagnosed on the basis of the structure of the egg masses (Wilson & Lee, 2005; see also Wilson, 2002). Rebecca Johnson has kindly provided us with additional data based on her as yet unfinished molecular and morphological research.

For the first of these two clades (the "black line group"), data on secondary metabolites are available for three species: the more basal *Chromodoris elisabethina* (**Photo 74**) and a more closely related pair of species, *Chromodoris lochi* (**Photo 75**) and *Chromodoris hamiltoni* (**Photo 76**). Latrunculins are nitrogenous macrolides that seem to be characteristic of this clade. It is believed that they are produced by symbionts of the sponges (personal communication from P. T. Murphy to Rudman & Bergquist 2007). Latrunculin-A (**Atlas 617**) was originally found in *Chromodoris elisabethina* (Okuda & Scheuer, 1985). Its name derives from one of the sponges that this slug eats, and from which it obtains the metabolite, originally identified as *Latrunculia magnifica*, but probably *Negombata* (Demospongiae: Poecilosclerida: Mycalina: Podospongiidae). It also occurs in

some sponges of the order Dictyoceratida and family Thorectidae. The latrunculin-A protects the nudibranch from predation by fish.

Chromodoris lochi, which, like its associated sponge, supposedly *Spongia mycofijiensis* (Demospongiae: Dictyoceratida: Spongiidae), but evidently *Petrosaspongia mycofijiensis* (Demospongiae: Dictyoceratida: Thorectidae), contains the sesquiterpenoid dendrolasin (**Atlas 346**) and latrunculin-A (**Atlas 617**) (Kakou, Crews & Bakus, 1987). The same species of nudibranch also contains the macrocyclic fatty acid lactones laulimalide (**Atlas 49**) and isolaumalide (**Atlas 50**) that derive from a sponge identified as *Hyattella* sp. (Demospongiae: Dictyoceratida: Spongiidae) (Corley, Herb, Moore, Scheuer & Paul, 1988). Animals of several other *Chromodoris* species were also observed feeding on the same sponge. *Chromodoris hamiltoni*, from South Africa, was found to contain both latrunculin-A (**Atlas 617**) and latrunculin-B (**Atlas 618**), a series of four chlorinated homoditerpenes (hamiltonins A-D) (**Atlas 425-428**) and a new sesterterpene, hamiltonin E (**Atlas 502**) (Pika & Faulkner 1995). In another study on specimens of the same species from Mozambique, there were found latrunculin-B (**Atlas 618**) and two new spongian diterpene lactones, 7β,11β-diacetoxy-16-oxospongian-17-al (**Atlas 423**), and 7β,11β-diacetoxy-16-oxospong-12-en-17-al (**Atlas 424**) (McPhail & Davies-Coleman, 1997).

In the second group the majority are Indo-West Pacific, but what appears to be an early branch occurs in the Atlantic. Specimens of *Chromodoris luteorosea* (**Photo 77**) from both Italy and Spain were found to contain the rearranged diterpenoids luteorosin (**Atlas 418**) and macfarlandin-A (**Atlas 417**) (Cimino, Crispino, Gavagnin & Sodano 1990; Gavagnin, Vardaro, Avila, Cimino & Ortea, 1991). (For a general review on diterpenoids in opisthobranchs, see Gavagnin & Fontana, 2000).The Italian specimens also contained aplysillins (**Atlas 419, 420**) similar to those that occur in the sponge *Aplysilla rosea* (Demospongiae: Dendroceratida: Darwinellidae), upon which the animal is suspected of feeding (Kazlauskas, Murphy, Wells & Daly, 1979). The Spanish specimens also contained a similar diterpenoid, norrisolide (**Atlas 384**). Some of these compounds had already been found from nudibranchs collected in the Eastern Pacific. The norditerpene acetate macfarlandin-A (**Atlas 417**), which resembles metabolites found in *Aplysilla sulphurea* (Demospongiae: Dendroceratida: Darwinellidae), was originally found in *Chromodoris macfarlandi* (**Photo 100**) (Molinski & Faulkner, 1986; Molinski, Faulkner, He, Van Duyne & Clardy, 1986). Norrisolide (**Atlas 384**) and similar compounds were found in *Chromodoris norrisi* (**Photo 84**) (Hochlowski, Faulkner, Matsumoto & Clardy, 1983). It resembles metabolites from *Aplysilla polyraphis* (Demospongiae: Dendroceratida: Darwinellidae) (Bobzin & Faulkner, 1989). Again, *Chromodoris marislae* (**Photo 78**) likewise contains rearranged diterpenoids derived from sponges (Hochlowski & Faulkner, 1981). This second group obviously specializes on sponges of the order Dendroceratida and family Darwinellidae, as has been emphasized by Rudman and Bergquist (2007).

Rearranged diterpenoids have also been found in *Chromodoris* from the Indian Ocean. De Silva, Morris, Miao, Dumdei and Andersen (1991) described several of them, such as shahamin K (**Atlas 396**), from the integument of *Chromodoris gleniei*, *Chromodoris geminus* (**Photo 79**), *Chromodoris annulata* (**Photo 80**), and "*Chromodoris inopinata*" (probably *C.. reticulata* or *C. tinctoria*) collected in Sri Lanka. *Chromodoris cavae* contains two others, chromodorolides A (**Atlas 394**) and B (**Atlas 395**) (Dumdei, De Silva, Andersen, Coudhary & Clardy, 1989; Morris, De Silva & Andersen 1991). *Chromodoris obsoleta* has been found to contain a series of spongian diterpenoids called dorisenones (**Atlas 407-409**) (Miyamoto, Sakamoto, Arao, Komori, Higuchi & Sasaki, 1996). "*Chromodoris inornata*" (subsequently reidentified as *C. orientalis*, but evidently *C. aspersa*) from Japan was found to contain toxic sesterterpenoids (Miyamoto, Sakamoto, Amano, Higuchi, Komori & Sasaki, 1992). Three of these were cytotoxic inorolides (**Atlas 485, 499, 500**),

six were scalarins, and two were scalarobutenolides. Miyamoto (2006) proposed that all of these molecules could be derived from a common precursor, cheilanthane, by different kinds of ring closure for each of the three classes of metabolites. Sesterterpenoids have also been found in "*Chromodoris funerea*" (*C. lineata*) (Carté, Kernan, Barrabee & Faulkner, 1986). Some display the scalarane skeleton, whereas others, such as luffariellins C and D (**Atlas 477, 478**), are monocyclic sesterterpenoids. A comparison of specimens from inside and outside a marine lake in Palau showed that the former sample contained only sesterterpenes, whereas the others contained a wide range of other metabolites, including polybrominated biphenyl esters. Evidently the animals are not dependent on a particular diet for their defensive metabolites (Kernan, Barrabee & Faulkner, 1988).

Specimens of *Chromodoris reticulata* (not *C. inopinata*) (**Photo 81**) from the South China Sea were separated into mantle and visceral portions and their metabolites identified (Carbone, 2007). The mantle was found to contain three diterpenoids with a spongian skeleton. 7-α-acetoxy-spongian-16-one (**Atlas 416**) is known from the sponge *Aplysilla rosea* (Demospongiae: Dendroceratida: Darwinellidae) and also occurs in *Chromodoris inopinata* and what may be a synonym for it, *C. obsoleta*. 7α-acetoxydendrillol-3 (**Atlas 415**) has also been found in *C. obsoleta*. Aplysioroseol-2 is known from sponges. Traces of it occur in the digestive gland and much higher concentrations in the mantle tissues, indicating that it is actively concentrated by the slug.

Chromodoris geometrica seems to be a mimic of porostome dorids of the family Phyllidiidae, as can be seen from **Photo 59**, in which this animal is shown crawling over a specimen of *Phyllidia pustulosa*. Specimens from Hainan were separated into mantle and visceral samples and their metabolites identified. Recovered were the rearranged diterpenoids macfarlandin-E, which is known from *C. macfarlandi*, dendrillolide-A (**Atlas 404**), known from a sponge, and norrisolide (**Atlas 384**), also known from *C. norrisi*.

The previous finding of spongian diterpenoids from "*Ceratosoma brevicaudatum*" (Ksebati & Schmitz, 1987), later identified as *Chromodoris epicuria*, further confirmed the typical *Chromodoris* chemical pattern (Ksebati & Schmitz, 1988).

What has been called "*Chromodoris petechialis*" has been found to contain spongiane-16-one (Karuso & Scheuer, 2002).

In general, then, the metabolites recovered from *Chromodoris* specimens are diterpenoids, but occasionally there are sesterterpenoids. These exceptions occur in two distinct clades, but the data now available do not tell us whether the sesterterpenoids occur in closely related animals in the second group. Latrunculins seem to occur in closely related animals that feed on a particular group of sponges, but the sponges are not closely related; rather they have the same metabolites. It seems likely that within the genus as a whole there is a trend in the direction of increased feeding specificity and the use of a smaller range of defensive metabolites. But this specialization has not been complete.

The remaining genera within the Chromodorididae tend to be more elongate than *Chromodoris*, and there seems to be a general trend toward the animals having somewhat higher bodies and a less extensive dorsum, so that they do not hug the substrate so closely. Such a configuration is etymologically expressed by the name of the genus *Hypselodoris*. A more extreme development of this tendency is seen in the genus *Ceratosoma*.

Ceratosoma

Ceratosoma has a distinctive body shape, as can be seen from **Photos 84** and **85**. The side of the foot is quite high and the notum is considerably reduced. Accompanying this development there are projections of the body in the vicinity of the gill, which are armed with repugnatorial glands.

The color pattern is such that it diverts the attention of potential predators away from the gill and toward the repugnatorial glands. It is easy to imagine how a fish nibbling at the nudibranch would get a dose of distasteful chemicals in its mouth. In *Ceratosoma brevicaudatum* these are said to be sesquiterpene furans and thiosesquiterpenes (Ksebati & Schmitz, 1988). There are also furanosesquiterpenoids in *C. trilobatum* (**Photo 84**) and *C. gracillimum* (**Photo 85**) (Mollo, Gavagnin, Carbone, Guo & Cimino, 2005; Wahidulla, Guo, Fakhr & Mollo, 2006), the main one being (-)-furodysinin (**Atlas 328**), which is both deterrent and toxic to fish. These compounds are known from sponges of the genus *Dysidea* (Demospongiae: Dictyoceratida: Dysideidae) (Cimino, De Stefano, Guerriero & Minale, 1975). These metabolites are concentrated in the repugnatorial glands. Surprisingly Grkovic, Appleton, and Copp (2005) recently reported having found a typical red algal metabolite, the terpenoid allalaurinterolacetate (**Atlas 282**), in *Ceratosoma amoena* from New Zealand and in an alga, *Hymenea variolosa*, upon which it was collected. They explained this anomaly by suggesting that although the mollusk is a carnivore it could also prey upon eggs of herbivores, such as *Aplysia*, that feed upon red algae containing halogenated sesquiterpenoids. Other possibilities might well be considered. For starters, some dorid nudibranchs that feed upon sponges also eat detritus (Kitting, 1991).

The remaining Chromodorididae consist of *Thorunna* and *Mexichromis* plus *Risbecia* and *Hypselodoris*. Provisionally we assume that these are a single clade with two branches, each made up of two genera. In general they feed upon sponges of the family Dysideidae in the order Dictyoceratida.

Thorunna

Thorunna daniellae, formerly placed in *Hypselodoris*, is the only representative of its genus for which data on secondary metabolites have been studied (Schulte & Scheur, 1982). It contains the sesquiterpenoid spiniferin-2 (**Atlas 332**), which originally was found in the sponge *Pleraplysilla spinifera* (Demospongiae: Dictyoceratida: Dysideidae). Nudibranchs of this genus are known to feed upon sponges of the family Dysideidae.

Mexichromis

Mexichromis (**Photo 87-88**) is now understood to be the sister group of *Risbecia* and *Hypselodoris*, from which it was separated. What is now called *Mexichromis porterae* (**Photo 88**) contains the sesquiterpenoids furodysinin (**Atlas 328**) and euryfuran (**Atlas 342, 343**), derived from *Dysidea amblia* (Demospongiae: Dictyoceratida: Dysideidae) (Hochlowski, Walker, Ireland & Faulkner 1982). Such metabolites are typical of *Risbecia* and *Hypselodoris*, which are the remainder of this clade and will now be considered.

Risbecia

Risbecia godfroyana, previously placed in *Hypselodoris*, was found to contain two furanosesquiterpenoids derived from the sponge *Dysidea fragilis* (Demospongiae: Dictyoceratida: Dysideidae) (Schulte, Scheuer & McConnell 1980). Similar metabolites occur in *Risbecia pulchella* from the Red Sea (**Photo 89**) (Ernesto Mollo, personal communication). **Photo 90** shows *Risbecia imperialis*.

Hypselodoris

Gosliner and Johnson (1999) based their phylogeny of *Hypselodoris* on morphology. They also analyzed the biogeography of the various lineages within the genus. They distinguished two

major clades. The first is an Indo-Pacific clade. Although this group is the more diverse of the two at the species level, data on its metabolites are sparse. Indeed metabolites have only been described from three species, *Hypselodoris nigrostriata, H. capensis*, and *H. maridadilus*. The other occurs in the Atlantic and the Eastern Pacific. The richer literature on the second group may have something to do with its accessibility to two major centers of research on marine natural products chemistry! Within the second group, *Hypselodoris orsini*, from the Mediterranean, is understood to have branched off before the rest of them. These form two clades, one largely Atlantic, the other from the Pacific coast of North America. We will treat them in that order.

Beginning with the Indo-Pacific clade, *Hypselodoris capensis* (**Photo 91**) contains terpenoids that would seem to be derived from an unidentified species of the sponge *Fasciospongia* (Demospongiae: Dictyoceratida: Thorectidae) (McPhail, Davies-Coleman & Coetzee 1998). The slugs were found to contain linear β-substituted furan sesterterpenes (**Atlas 471-473**) as well as sesquiterpenes such as nakafuran-8 (**Atlas 338**). *Hypselodoris maridadilus* (**Photo 92**) contains nakafurans, which are furanosesquiterpenes, derived from the sponge *Dysidea fragilis* (Demospongiae: Dictyoceratida: Dysideidae) (Schulte, Scheuer & McConnell, 1980). *Hypselodoris nigrostriata* (**Photo 93**), which ranges from the western Indian Ocean to Hong Kong contains the sesquiterpenoid furodysinin (**Atlas 328**), which also occurs in sponges of the genus *Dysidea* (Fontana, Ciavatta, D'Souza, Mollo, Naik, Parameswaran, Wahidulla & Cimino, 2001). It is the major metabolite in the repugnatorial glands of these beautiful animals.

Turning now to the Atlantic/Pacific clade, *Hypselodoris villafranca* (**Photo 94**), recorded as *H. gracilis* by Cimino, De Stefano, De Rosa, Sodano, and Villani (1980) and as *H. villafranca* by Avila, Cimino, Fontana, Gavagnin, Ortea and Trivellone (1991), was found to contain furanosesquiterpenoids (**Atlas 347-349**) derived from *Dysidea fragilis* (Demospongiae: Dictyoceratida: Dysideidae) and typical of that genus. The metabolites are concentrated in the digestive gland, mucus, and mantle dermal formations.

Hypselodoris orsini, (**Photo 95**) formerly called *H. coelestis*, has often been misidentified as *Glossodoris tricolor*. It contains sesterterpenoids rather than sesquiterpenoids, making it one of the occasional exceptions (together with *H. capensis* and *H. ghiselini*) to the rule in its genus (Cimino, Fontana, Giminéz, Marin, Mollo, Trivellone & Zubia, 1993). It feeds upon the sponge *Cacospongia mollior* (Demospongiae: Dictyoceratida: Thorectidae), which is rich in scalaradial (**Atlas 486**). It then modifies this metabolite by selective transformations including the oxidation of deoxoscalarin (**Atlas 483**) to 6-keto-deoxoscalarin (**Atlas 489**), which gets transferred to the mantle dermal formations (Cutignano & Fontana, personal communication). Because this modified metabolite seems to be biologically inactive, it has been speculated that the mantle dermal formations are serving as a kind of excretory organ (Avila & Dufort, 1996).

A wide range of furanosesquiterpenoids have been derived from *Hypselodoris cantabrica*, which is the sister group of the remaining species to be discussed (Fontana, Avila, Martinez, Ortea, Trivellone & Cimino, 1993).

The remaining species of *Hypselodoris* are thought to form a monophyletic unit with two major branches. The first of these contains *Hypselodoris bayeri* and the closely related *Hypselodoris zebra* and *Hypselodoris picta* (formerly *Hypselodoris webbi*).

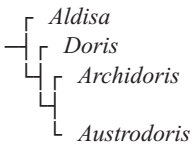
Hypselodoris zebra and *Hypselodoris picta* are closely related. *Hypselodoris zebra* from the Pacific contains furanosesquiterpenes that also occur in the sponge *Dysidea etheria* (Demospongiae: Dictyoceratida: Dysideidae) (Grode & Cardellina, 1984). *Hypselodoris picta* (**Photo 97**) contains furanosesquiterpenoids (Fontana, Giménez, Marin, Mollo & Cimino, 1994). In this paper it was rigorously proved that the mollusk is able to transfer sponge metabolites into mantle dermal formations that are strategically placed along the border of the mantle. Related to

them is *Hypselodoris bayeri*, which again contains furanosesquiterpenoids (Fontana, Trivellone, Mollo, Cimino, Avila, Martinez & Ortea, 1994).

The remaining branch consists mainly of Eastern Pacific animals (Hochlowski, Walker, Ireland & Faulkner, 1982). *Hypselodoris agassizi* (**Photo 98**) is thought to be the sister group of *Hypselodoris californiensis* and *Hypselodoris ghiselini*. *Hypselodoris agassizi* was found to contain a sesquiterpene furan, agassizin (**Atlas 327**). Specimens of *Hypselodoris californiensis* (**Photo 99**) from the Gulf of California contained the sesquiterpenes dendrolasin (**Atlas 346**) and nakafuran 8 (**Atlas 338**), whereas specimens from San Diego contained furodysinin (**Atlas 343**), euryfuran (**Atlas 342**) and pallescensin A (**Atlas 344**). Nakafuran-8 and nakafuran-9 (**Atlas 338, 349**) have been found in sponges of the genus *Dysidea* (Demospongiae: Dictyoceratida: Dysideidae). *Hypselodoris ghiselini* (**Photo 100**) contained a diterpene epoxide called ghiselinin (**Atlas 396**) and also the sesquiterpenoids dendrolasin (**Atlas 346**), nakafuran-9 (**Atlas 349**) and a methoxy butenolide. *H. orsini*, *H. capensis* and *H. ghiselini* are the three known exceptions to the rule that *Hypselodoris* contains only furanosesquiterpenoids. It would appear that *Hypselodoris ghiselini* is less specialized in its use of a range of metabolites than the generality of its genus. If it occupied a more basal position in the tree, one would suspect that this euryphagy is primitive; but, given its close relationship to trophic specialists, this condition is probably secondary. The similarities in coloration among *Hypselodoris agassizi*, *Hypselodoris californicus*, and *Hypselodoris ghiselini* suggest a mimetic complex among these aposematic organisms. Some specimens of the cephalaspidean *Navanax inermis* collected in the same area also display a similar pattern, as does a flatworm.

The remaining dorids constitute a single lineage. They have been divided into two main groups, which Valdés (2002) calls the families Dorididae and Discodorididae.

The Dorididae for which we have chemical data are *Aldisa*, which is basal to the remainder (and probably the sister group of *Cadlina*), *Doris*, and two genera which are probably quite closely related: *Archidoris* and *Austrodoris* (of which *Anisodoris* is considered a synonym). The relationships between them are shown in the following tree:



Aldisa sanguinea contains steroids (**Atlas 572, 573**), one of which repels predators (Gustafson & Andersen, 1982, 1985). They occur only in the integument, not in the digestive gland, and the sponge upon which it feeds has neither of those found in the nudibranch, but instead contains the non-repellant steroid cholestenone. Evidently steroids from the food are modified by the slug. *Aldisa smaragdina*, from the Mediterranean contains a structurally similar steroid which could have the same steroid (**Atlas 574**) precursor (Gavagnin, Ungur, Mollo, Templado & Cimino, 2002). However, 3-ketosteroids were completely absent from the sponge on which the animal lives. **Photo 101** shows this animal well concealed on the sponge, which has been identified as *Phorbas fictitius* (Poecilosclerida: Myxillina: Hymedesmiidae).

Doris verrucosa (**Photo 103**) contains toxic esters called verrucosins (**Atlas 529, 530**). These are diterpenoic acid diglycerides (Cimino, Gavagnin, Sodano, Puliti, Mattia & Mazzarella, 1988; Avila, Ballesteros, Cimino, Crispino, Gavagnin & Sodano, 1990; Gavagnin, Ungur, Castelluccio & Cimino, 1997; De Petrocellis, Orlando, Gavagnin, Ventriglia, Cimino & Di Marzo, 1996). The verrucosins do not occur in the sponge, *Hymeniacidon sanguinea* (Demospongiae: Halichondrida: Halichondridae), upon which the nudibranch feeds, nor do they occur in the digestive glands. Initial

results suggesting *de novo* biosyntheses were confirmed experimentally in a paper by Gustafson and Andersen (1985). More direct evidence was provided by experiments with labeled glucose and pyruvate, showing how they are incorporated in the molecules (Fontana, Tramice, Cutignano, D'Ippolito, Renzulli & Cimino, 2003). *Doris verrucosa* also biosynthesizes an unusual nucleoside (**Atlas 609**), which is an analog of methylthioadenosine, and uses it defensively in its eggs (Cimino, Crispino, De Stefano, Gavagnin & Sodano, 1986; 2877; Porcelli, Cacciapuoti, Cimino, Gavagnin, Sodano & Zappia, 1989).

The same nucleoside has been found in animals from Brazilian waters that would appear to be of the same species (Granato, Berlink, Magaljaes, Scheffer, Ferreira, De Sanctis, De Freitas, Hajdu & Migotto, 2000). In *Doris odhneri* (**Photo 102**) and *D. montereyensis* (**Photo 104**), reported as the synonymous genus *Archidoris* (Anderson & Sum, 1980; see also Gustafson & Andersen 1985), some terpenoid acid glyceride esters (**Atlas 523-526; 535-537**) were found. *De novo* synthesis of such glycerides was established by injection of labeled mevalonate (Gustafson, Andersen, Chen, Clardy & Hochlowski, 1984). Feeding experiments with stable isotopes confirmed these results (Graziani, Andersen, Krug & Faulkner, 1996). In addition, biosynthesis was proven for the sesquiterpenoid glycerides (**Atlas 527, 528**) from *Doris tanya* (as *Sclerodoris tanya*) (**Photo 105**). This animal was found to contain sesquiterpenoids linked to glycerol in the same way as the diterpenoids found in other Dorididae (Krug, Boyd & Faulkner, 1995). The presence of tanyolide A (**Atlas 527**) and tanyolide B (**Atlas 528**) in the mantle but not in the digestive glands suggested *de novo* synthesis. One of these compounds, tanyolide B, was subsequently shown conclusively to be synthesized *de novo* by injection of labeled 2-^{13}C mevalonolactone (Graziani, Andersen, Krug & Faulkner, 1996).

Doris pseudoargus, originally misidentified as *Archidoris tuberculata*, was found to have acylglycerols (**Atlas 532**) esterified in position 2-*sn* by diterpenoid acids (Cimino, Crispino, Gavagnin, Trivellone, Zubia, Martinez & Ortea, 1993; Zubia, Gavagnin, Crispino, Martínez, Ortea & Cimino 1993). Their presence in the integument but not the digestive gland again was strongly suggestive of *de novo* synthesis. Huysecom, Van de Vyver, Braekman and Daloze (1999) characterize this species as being euryphagous and preferring to feed upon non-toxic sponges. This again makes sense in an animal that relies upon metabolites that it biosynthesizes rather than obtaining them from food. Soriente, Sodano, Reed, and Todd (1993) independently described the same diacylglycerol, but with the diterpenoid erroneously linked at position 1-*sn*, from what they called *Archidoris pseudoargus*. They found that it was toxic to fish.

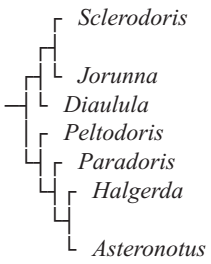
In addition, the same pair of diterpenoid glycerides (**Atlas 536, 537**), previously described from *Doris montereyensis* (Gustafson & Andersen 1985) was detected. Apparently identical compounds (**Atlas 533, 534**) differing only in stereochemistry of the diterpenoid were found in *Anisodoris fontaini*, discussed originally as *Anisodoris carvi*. The absolute stereochemistry of the diterpenoid is the opposite (Gavagnin, Ungur, Castelluccio, Muniain & Cimino 1998).

Austrodoris, as the name suggests, is a southern genus and much of the work on it has been done in Antarctica and adjacent regions. It is now considered a synonym of *Doris*, and we will follow that innovation here. *Doris kerguelenensis* was found to contain diterpenoic acid glycerides (**Atlas 552-556**) that resemble those of other *Doris* species, which are known to be synthesized *de novo* (Davies-Coleman & Faulkner 1991). According to a personal communication to these authors the animals feed on sponges of the class Hexactinellida, but no metabolites were found in the sponges, again suggesting *de novo* synthesis. Wägele (1989) confirms the observation that the slug sits on hexactinellids (*Rossella* and *Scolymastra*). On the other hand, Barnes & Bullough (1996) say that this species is a specialist on *Dendrilla antarctica*, a sponge of the class Demospongiae (Dendroceratida: Darwinellidae). Evidently the association is not obligatory as had been suspect-

ed. Gavagnin, Trivellone, Castellucio, Cimino and Cattaneo-Vietti (1995) described a glyceryl ester of a halimane diterpenoic acid (**Atlas 558**) from the integument of this species. The compound is probably biosynthesized *de novo* (Gavagnin, Fontana, Ciavatta & Cimino, 2000). The metabolites do not occur in the gut, pointing to the same conclusion. The echinoderm *Odontaster validus* is deterred by diterpene diacylglycerides and monoglycerides of regular fatty acids that occur in the mantle tissue (Iken, Avila, Fontana & Gavagnin, 2002). Nor-sesquiterpenes (**Atlas 248, 249**) have been characterized (Gavagnin, Carbone, Mollo & Cimino, 2003a) and confirmed by synthesis (Gavagnin, Carbone, Mollo & Cimino (2003b). They turn out to have the same stereochemistry as similar metabolites from other dorids.

Thus the animals that we have just surveyed under the rubric of Doridiidae generally contain terpenoid esters in the integument, and there is much evidence for these being biosynthesized *de novo*. They also frequently contain other metabolites, some of which are obtained from food. The earliest branch of this group for which we have data, *Aldisa*, does not fit this pattern, but instead contains steroids perhaps derived from food, but modified. We defer considering whether this is what the common ancestor did, or whether it is a specialization of this genus.

Let us now turn to the other branch, or Discodoridiidae of Valdés (2002). These are the remainder of the dorids and have been broken down into two clades, as represented in the following tree:



We will first consider the clade that has been diagnosed on the basis of possessing caryophyllidia, which are specialized sensory organs of unknown function on the dorsal surface (Valdés & Gosliner, 2001). The genera for which data on secondary metabolites are available are *Diaulula* and *Jorunna*.

Diaulula sandiegensis (**Photo 106**) was found to contain nine chlorinated polyacetylenes (**Atlas 3-11**) (Walker & Faulkner, 1981). A variety of sponges were found in the animal's gut, but none of these contained acetylenes. Later, however, these polyacetylenes plus two new ones were recovered from the sponge *Haliclona lunisimilis* (Demospongiae: Haplosclerida: Haplosclerina: Chalinidae) (de Jesus & Faulkner, 2003). However, the absence of ketones in the sponge led the authors to suggest that the mollusk is able to synthesize these compounds by oxidation of the corresponding alcohols. Acetylenes are rare in marine organisms though they sometimes occur in algae, sponges and corals. From a sample taken in a more northerly part of the range, two new steroids, diaulusterols A and B (**Atlas 577, 578**), which resemble various hormones, were obtained from skin extracts (Williams, Ayer & Andersen, 1968). There is compelling experimental evidence for biosynthesis of the polyacetate portion of the molecule, but not for the steroid part (Kubanek & Andersen, 1999).

Jorunna funebris, shown in **Photo 107**, was found to contain isoquinoline quinones (Kubo, Kitahara & Nakahara, 1989). These were said to come from an otherwise unidentified sponge of the genus *Xestospongia* (Demospongia: Haplosclerida: Petrosina: Petrosiidae). From the integument and mucus of animals of the same species there was also isolated an isoquinoline alkaloid, jorumycin (**Atlas 629**), similar to ones known from bacteria, sponges and tunicates (Fontana,

Cavaliere, Wahidulla, Naik & Cimino, 2000). The nudibranch was taken from a sponge of the genus *Oceanapia* (Demospongia: Haplosclerida: Petrosina: Phleodictyidae), which did not contain such metabolites, although it did contain the monomeric isoquinolines, which may be breakdown products of jorumycin. Specimens from Thailand contained three new isoquinoline alkaloids (**Atlas 630**) together with previously known ones (Charupant, Suwanborirux, Annuoypol, Saito, Kubo & Saito, 2007). A derivative of one of these alkaloids, Zalapsis®, is now undergoing clinical trials as an anticancer drug.

The remaining Discodorididae lack caryophyllidia but have a notched anterior end of the foot and digitate oral tentacles. Taken in order of branching, the genera of interest here are: *Peltodoris*, *Paradoris, Asteronotus*, and *Halgerda*.

In *Peltodoris atromaculata* (**Photo 110**) a variety of sterols and high-molecular-weight polyacetylenes (**Atlas 12, 13**) have been found, all supposedly derived from the sponge *Petrosia ficiformis* (Demospongia: Haplosclerida: Petrosina: Petrosiidae) (Castiello, Cimino, De Rosa, De Stefano, Izzo & Sodano, 1979; Castiello, Cimino, De Rosa, De Stefano & Sodano, 1980; Cimino, de Stefano, De Rosa, Sodano & Villani, 1980; Guo, Gavagnin, Trivellone & Cimino, 1994). The steroid petrosterol (**Atlas 569**) was present in both the sponge and the nudibranch. Behavioral experiments using Y-tubes showed that the nudibranchs prefer the sponge and also an aqueous extract of it, but not extracts without the lipid fraction. On the other hand Avila and Dufort (1996) point out that the metabolites accumulate only in the gut, and therefore are not defensive. Preference of *Peltodoris atromaculata* for certain haplosclerid sponges has also been shown on the basis of field observations and fecal analysis (Gemballa & Schermutzki, 2004). The sponges fed upon turn out to be two species of *Petrosia*, not just one, and *Haliclona fulva* (Demospongiae: Haplosclerida: Haplosclerina: Chalinidae). The former are more common, and therefore more of them are consumed, but the latter are preferred. Both contain the acetylenes, and these also occur in other sponges and the nudibranchs that feed upon them. Gemballa and Schermutzki (2004) conclude that the nudibranchs are defended by their spicules, and have specialized on toxic sponges as a source of food, not defensive metabolites. It is surprising that both of the sponges contain very unusual polyacetylenes with a long alkyl chain with 46 carbon atoms (personal communication from Letizia Ciavatta).

Peltodoris nobilis (**Photo 109**) contains a pharmacologically active N-methylpurine riboside (**Atlas 615**) that was extracted from the digestive glands and resembles molecules known from a sponge (Fuhrman, Fuhrman, Kim, Pavelka & Mosher, 1981; Fuhrman, Fuhrman, Nachman & Mosher, 1981; Kim, Nachman, Pavelka, Mosher, Fuhrman & Fuhrman, 1981). It resembles the compound (**Atlas 609**) discussed above from *Doris verrucosa*. Kitting (1981) studied the feeding behavior in relation to chemical defense in considerable detail. The animal eats some detritus, in addition to feeding selectively on a variety of chemically-defended sponges. The sponges upon which the nudibranch feeds are repugnant to both dorids and dendronotaceans. Bioassays of nudibranchs that had been starved showed a decline in pharmacological activity, indicating that the metabolite came from the food and was not biosynthesized, unlike the similar compound in *D. verrucosa*.

Paradoris indecora was formerly placed in the genus *Discodoris*. It lives perfectly camouflaged on the sponge *Ircinia variabilis* and also occurs on *Ircinia fasciculata* (Demospongiae: Dictyocerata: Irciniidae). From the sponges it obtains the furanosesterterpenoids fasciculatin (**Atlas 471**), variabilin (**Atlas 472**) and palinurin (**Atlas 473**) (Marin, Belluga, Scognamiglio & Cimino, 1997). The terpenoids are sequestered by the nudibranch in dorsal tubercles, and released as a white secretion when the animals are disturbed.

Asteronotus and *Halgerda* are more closely related to each other than either is to any other lin-

eage (Fahey & Gosliner 2001). Specimens of *Asteronotus caespitosus* (**Photo 108**) from the western Pacific display an illuminating pattern of geographical variation (Fahey & Garson, 2002). The metabolites found (**Atlas 174-176, 230, 231, 631, 632**) were ones characteristic of the sponge *Dysidea herbacea* (Demospongiae: Dictyoceratida: Dysideidae) from the same region (Norton, Croft & Wells, 1981; Handayani, Edrada, Proksch, Wray, Witte, Van Soest, Kunzmann & Soedarsono, 1997). Metabolites from the digestive system included cytotoxic polybrominated diphenyl ethers and a new hexachlorinated alkaloid. These, however, did not occur in the integument. Evidently, although tolerated, they are not used defensively. Two sesquiterpenes previously unknown from mollusks, dehydroherbadysidolide and spirodysin (**Atlas 230-231**), were found in the integument of specimens from some areas. Because the arrays of metabolites differed among specimens, it was concluded that the animals obtained them from their food, and did not biosynthesize them *de novo*.

For *Halgerda*, illustrated by two species in **Photos 111** and **112**, trees based on morphological and molecular evidence are available (Fahey & Gosliner, 2001). Seven species have been surveyed for secondary metabolites (Fahey & Carroll, 2007). None of them show the kind of metabolites noted for *Asteronotus*, and indeed all but two are well camouflaged and seem to have no defensive metabolites. Both *Halgerda gunnessi* and *Halgerda aurantiomaculata* on the other hand would seem to have warning coloration, and extracts of them are cytotoxic. These two species are not closely related, and they have quite different metabolites. *Halgerda aurantiomaculata*, which has been most intensively studied, contains several alkaloids (**Atlas 621-625**), some of which are known from sponges. These are 1) halgerdamine (**Atlas 621**), a tryptophan derivative, 2) C2-α-*D*-mannosylpyranosyl-*L*-tryptophan (**Atlas 622**), 3) trigonellin (**Atlas 623**), 4) esmodil (**Atlas 625**), and 5) zoanemonin (**Atlas 625**) (in high concentration, known from sponges). The second of these compounds is known from human tryptophan metabolism, but otherwise is known only from tunicates. *Halgerda gunnessi* contains mixtures of acylated tetrasaccharides. The genus is a good example of the continued adaptive radiation of tropical dorid nudibranchs.

By way of summary, let us consider the picture that emerges from an examination of the trees presented in this chapter. The relationships shown in the diagrams are those that have been followed in the text. However, it should be emphasized that the exact branching sequences are questionable. The dorid nudibranchs generally feed upon sponges, and there is good reason to believe that their common ancestor was no exception. Shifts away from spongivory have taken place in several lineages within the group, all of which branched off before the common ancestor of the Cryptobranchia. *Bathydoris* has evidently expanded its range of food items so as to become a generalist scavenger, though it continues to use sponge metabolites as a means of defense. It is an inhabitant of deeper and colder waters in which there is a scarcity of food, and such a shift in feeding habits is quite common under such circumstances. *Hexabranchus*, on the other hand, dwells in the shallow, tropical waters where predation is a serious problem. It is a common animal and seems to flourish partly because it has pressed so many sponge-derived defensive metabolites into service.

The notion that Phanerobranchia is a paraphyletic grade consisting of three separate branches is expressed in the diagram. One might wonder whether, as often happens, the paraphyly is the product of an insufficient number of synapomorphies being present to hold a monophyletic group together. But taking the branching sequences at face value we get some evident parallelism. In the first lineage, Polyceratidae, we have animals that are protected by alkaloids, but these derive, not from sponges, but from bryozoans, tunicates and other animals, and in at least one case through *de novo* synthesis. The Aegiridae, which are the next lineage, feed upon sponges, but these are calcareous sponges, and they derive alkaloids from them. Finally the Suctoria are known to have ter-

penoids derived from Bryozoa. In an earlier work (Cimino & Ghiselin, 1999) we suggested that the phanerobranchs perhaps took to feeding upon calcareous sponges with alkaloids, and then shifted to various other animals with alkaloids. This scenario is not clearly supported by the tree that we have followed and the sequence proposed earlier would have to involve some reversals or parallelisms. We leave this issue open.

The cryptobranch dorids are sponge feeders, with a few isolated exceptions, and it seems likely that the common ancestor derived defensive terpenoids from Demospongiae. The first branch, Porostomata, with its peculiar feeding mechanism, shows an interesting divergence. On the one hand we have the Dendrodorididae, some of which are rather euryphagous and have been emancipated from their original metabolites by the capacity for *de novo* synthesis of terpenoids. The available sample, however, may not be representative of the group. The Phyllidiidae, on the other hand, are remarkably adept at utilizing isocyanide terpenoids defensively.

In the Chromodorididae a definite trend is apparent, such that the animals in each genus tend to specialize on a limited range of terpenoids.

With the exception of the basal branch, *Aldisa*, the Doridiidae that have been studied make use of defensive metabolites that are terpenoid glycerides. The majority of the terpenoids are typical sponge metabolites. However, it has been proven by synthetic experiments that the mollusks biosynthesize both the terpenoid skeleton and the glycerols. This is an evolutionary innovation unique among nudibranchs.

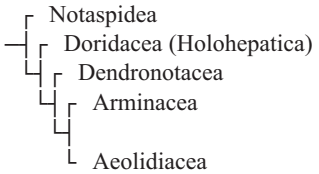
In Discodorididae, which are the remaining dorids, there are quite a variety of sponge-derived metabolites. The presence of steroids and acetylenes in the skin of *Diaulula* and *Peltodoris* makes one wonder if these organisms are quite accurately placed in the tree.

The dorids are noteworthy for having evolved the capacity for *de novo* synthesis of metabolites upon several occasions. This development has taken place in quite isolated parts of the tree, and a wide diversity of metabolites are synthesized. Thus we find *Bathydoris* perhaps biosynthesizing a sesquiterpene known from sponges, *Triopha* and *Polycera* biosynthesizing an alkaloid characteristic of bryozoans, *Acanthodoris* biosynthesizing sesquiterpenoids, *Dendrodoris* and *Doriopsilla* biosynthesizing sesquiterpenoids characteristic of sponges, *Cadlina*, *Archidoris*, *Anisodoris*, and *Doris* making diterpenoic acid diglycerides, and *Doris tanya* making diterpene and sesquiterpene glycerides. Given that only a small proportion of the dorids have been sampled, it is obvious that many more examples await discovery.

Chapter XI

Other Nudibranchs

The relationships among the four major groups of nudibranchs are suggested by the following diagram:



Like the dorids, the remaining nudibranchs constitute a monophyletic group. It was named

Cladohepatica by Thiele (1931) on the basis of the digestive gland being branched rather than existing as a single mass as in Holohepatica. It seems likely that any exception to this diagnostic feature is due to a reversion to a more ancestral state. The branching correlates with a shift to feeding on cnidarians, which are protected largely by their stinging capsules (cnidae or nematocysts) but also have chemical defenses. One point of departure for thinking about such matters is the fact that in addition to nematocysts, cnidarians of the subclass Octocorallia also have both chemical defense and spicules, therefore resembling sponges in some important respects. Sometimes the nematocysts of the prey are preempted by the nudibranch and used defensively, suggesting the kind of organelle symbiosis that occurs in sacoglossans. A few of the cladohepatic nudibranchs no longer feed upon cnidarians, and how they defend themselves provides some very instructive material for evolutionary biology.

Traditionally, Cladohepatica has been subdivided into three groups: Dendronotacea, Arminacea, and Aeolidiacea. The names derive from those of three genera: *Dendronotus, Armina*, and *Aeolis* respectively. At present there is good evidence that Arminacea and Dendronotacea are paraphyletic groups, but revising the system is work in progress.

Part 1. Dendronotacea

Data on chemical defense in Dendronotacea are limited to two families, one of which is thought to be relatively primitive (Tritoniidae) and the other highly modified (Tethyidae). We begin with the Tritoniidae. These animals feed upon octocorals. Whether or not this is the original food is debatable. Data on secondary metabolites are available for only three species: *Tritonia hamnerorum*, *Tochuina tetraquetra*, and *Tritoniella belli*. According to the only available tree, the family is paraphyletic and even the genus *Tritonia* is polyphyletic (Smith, 2005). Although the tree is, as usual, somewhat provisional, the first species is very distantly related from the other two, which are close relatives.

Tritonia hamnerorum, shown on a sea-fan in **Photo 114**, has been found feeding on what appear to be more than one species of the sea-fan genus *Gorgonia* (Gosliner & Ghiselin, 1987; Cronin, Hay, Fenical & Lindquist 1995). The animals have a rather camphor-like smell, and contain, sequester and concentrate julieannafuran (**Atlas 319**), a furano germacrene sesquiterpenoid. In feeding experiments, fish were found not to attack intact nudibranchs.

Tochuina tetraquetra (**Photo 113**) feeds upon, and obtains metabolites from the soft coral *Alcyonium* sp., recorded as *Gersemia rubiformis* by Williams and Andersen (1987). The slugs and the corals from one location were found to contain two new cuparane sesquiterpenoids, tochuinyl acetate (**Atlas 218**) and dihydrotochuinyl acetate (**Atlas 219**) previously unknown from soft corals. The slugs also contained the diterpenoids rubifolide (**Atlas 354**), pukalide (**Atlas 355**) and ptilosarcenone (**Atlas 356**). (Pukalide has been mentioned above as a constituent of soft corals). Slugs from another location contained ptilosarcenone and an analogue of it.

Tritoniella belli, from Antarctic waters, mainly feeds on the octocoral *Clavularia frankliniana*. It contains the glyceride ester chimyl alcohol (**Atlas 522**), which provides protection from sea stars (McClintock, Baker, Slattery, Heine, Bryan, Yoshida, Davies-Coleman & Faulkner, 1994). The egg masses were found to be eaten by some predators, but not by others (McClintock & Baker, 1997; Bryan, McClintock & Baker, 1998).

In the family Tethyidae the anterior portion of the body has been modified so as to form a kind of cowl or net that is spread out and used to catch small crustaceans and perhaps other animals. **Photo 115** gives the example of *Tethys fimbria* with its net-like head on the left side of the picture. Several of the cerata on the left side of the body have autotomized and some of them are regener-

ating. These animals have shifted to a new food source, one that does not contain defensive metabolites. The metabolites that they use defensively are biosynthesized *de novo*. However, similar metabolites do occur in Octocorallia. These are used defensively, so it seems likely that the slugs' ancestors obtained defensive metabolites from food.

Tethys fimbria has been found to contain prostaglandin lactones (**Atlas 15-24**) (Cimino, Spinella & Sodano, 1989; Di Marzo, Cimino, Sodano, Spinella & Villani, 1990; Di Marzo, Cimino, Crispino, Minardi, Sodano & Spinella 1991; Cimino, Crispino, Di Marzo, Sodano, Spinella & Villani, 1991; Cimino, Crispino, Di Marzo, Spinella & Sodano, 1991; Marin, Di Marzo & Cimino 1991). Prostaglandins and other eicosanoids are common components of animal tissues and it is hardly surprising that the nudibranchs biosynthesize these compounds *de novo*. However, prostaglandins occur as defensive metabolites in many octocorals. It is likely that *Tethys* is descended from ancestors that fed upon such octocorals and used prostaglandins derived from food defensively. When the switch to a different food supply took place, metabolites produced by the nudibranch itself provided a substitute. Prostaglandins occur in the cerata of the nudibranch, which are readily autotomized. Early naturalists mistook the autotomized cerata for flatworms. The cerata regenerate readily, and prostaglandins stimulate the regeneration.

From *Melibe viridis* (**Photo 116**), an anatomically similar nudibranch of the same family but a different genus, one of the same eicosanoids, prostaglandin E_2-1,15-lactone (**Atlas 17**), has recently been isolated (Mollo, Gavagnin, Carbone, Castellucio, Pozone, Roussis, Templado, Ghiselin & Cimino, 2008). Its distribution in the body suggests *de novo* synthesis, but this has not been confirmed experimentally. In certain other species of the genus *Melibe* there would seem to be no defensive eicosanoids. Instead, there are terpenoids. *Melibe fimbriata* is said to have a fruity smell (Thompson & Crampton 1984), but its chemistry has not been studied. We have good information about secondary metabolites in *Melibe leonina*. The repugnatorial glands of this species have also been studied (Bickell-Page, 1991). The defensive compound, which again has a fruity odor, is concentrated in these glands. It is a degraded terpenoid, 2,6-dimethyl-5-heptenal (**Atlas 186**) (Ayer & Andersen, 1983). There is also the corresponding 2,6-dimethyl-5-heptenoic acid (**Atlas 187**), which lacks the fruity smell. Geographical distribution showed the presence of the terpenoids to be invariant, indicating the likelihood of *de novo* synthesis. Experimental work showed that labeled acetate was incorporated into the molecule in a way that is consistent with the mevalonic acid pathway (Barsby, Linington & Andersen, 2002). As already noted, terpenoids are often defensive metabolites in Octocorallia. In this case the scenario would be much the same as for the other genus, but the evolution of the biosynthetic capacity would be somewhat more difficult.

Part 2. Arminacea

In the Euarminacea, *Armina maculata* was found to contain a cembranoid and briarane diterpenoid (-)-verecynarmin-A (**Atlas 359**) (Guerriero, D'Ambrosio & Pietra, 1987, 1988) and similar compounds (**Atlas 360, 361**) supposedly derived from the pennatulacean *Veretillum cynomorium* (Octocorallia) upon which the nudibranchs feed (Guerriero, D'Ambrosio & Pietra, 1990). These are quite similar to metabolites that have been recovered from sponges. From *Armina babai* a ceramide (**Atlas 612**) has been recovered (Ishibashi, Yamaguchi & Hirano, 2006). This metabolite had previously been isolated from the gorgonian *Acabaria undulata* (Shin & Seo, 1995). In a different genus but the same family, *Dermatobranchus ornatus* (**Photo 117**) contains eunicellin diterpenoids that are also found in gorgonians, such as *Calicogorgia*, *Astrogorgia*, and *Muricella* (Carbone, 2007). *Dermatobranchus otome* was found to contain three new sesquiterpenoids (**Atlas 232-234**) of unknown origin (Ishibashi, Yamaguchi & Hirano, 2006). In *Leminda millecra* (**Photo**

118) four sesquiterpenes (**Atlas 220-223**) that resemble those of sponges were discovered (Pika & Faulkner, 1994). However, the gut contained spicules from the soft corals *Alcyonium* and *Drita*. Many sesquiterpenoids typical of gorgonians and seven triprenyl quinones and hydroquinones have subsequently been found (McPhail, Davies-Coleman & Starmer, 2001). The main metabolites occur in the gorgonian *Leptogorgia palma*, upon which the nudibranch has often been seen feeding.

Janolus cristatus, which may be closer to the other cladohepatic groups than to Euarminacea, feeds upon Bryozoa and contains janolusimide (**Atlas 600**), a lipotripeptide (Sodano & Spinella, 1986; Cimino, De Rosa, De Stefano & Sodano, 1986). **Photo 119** shows this animal with its pair of rhinophores and many dorsal processes that superficially resemble those of our next group (Aeolidiacea). When arminaceans of the genus *Dirona* feed upon bryozoans, the prey respond by increasing the production of metabolites (Harvell, 1984).

Part 3. Aeolidiacea

The aeolid nudibranchs are the last group to be treated. These animals feed mostly on cnidarians, though there are a few scattered exceptions (some eat eggs and some eat bryozoans). The characteristic feature of cnidarians is their cnidae, or stinging capsules. These may defend them from predators, including aeolid nudibranchs, which are famous for turning the cnidae against their own enemies (Edmunds, 1966). Such cleptocnidae, as they are called, may collect in special organs at the tips of the cerata. **Photo 120** shows a specimen of *Flabellina pedata*, an aeolid nudibranch on a colony of hydrozoans with their tentacles extended. As can be seen from the specimen of *Flabellina affinis* shown in **Photo 121**, *Calorica indica* shown in **Photo 128**, and *Godiva quadricolor* shown in **Photo 126**, the cerata are positioned in a manner that enhances their effectiveness. Often the tip of each ceras is brightly and conspicuously colored. The nudibranch's behavior may also help. The cerata may bristle when the slug is attacked (Millen & Hamann, 1992). The tips may converge upon an attacking predator. Cerata are sometimes autotomized, and when they are, they may writhe about, providing a distraction. In spite of such evidence it has occasionally been maintained that the cnidae do not function defensively and are merely excreted at the tips of the cerata (Streble, 1968). This interpretation goes too far.

Where the cnidae of the prey are large and highly toxic, the cleptocnidae can present a formidable defense mechanism, as is the case with the pelagic nudibranch *Glaucus* (**Photo 122**), which feeds upon the "man of war" jellyfish *Physalia* (Thompson & Bennett, 1969). However, the cnidae, even within a single animal, are not all of the same size and toxicity. In some cnidarians the cnidae are small and the animals mainly depend upon chemicals for defense. The aeolid nudibranchs that feed upon them likewise have other defense mechanisms. The assumption that the cnidae provide adequate defense from predators may be partly responsible for the small number of studies that have been carried out on the secondary metabolites of aeolid nudibranchs. It is known, however, that cnidarians use a combination of cnidae and secondary metabolites in defending themselves from predators (Stachowicz & Lindquist, 2000).

Secondary metabolites have been found in hydrozoans and in the aeolid nudibranchs that feed upon them. The hydroids *Eudendrium rameum*, *Eudendrium racemosum* and *Eudendrium ramosum* contain modified steroids (**Atlas 570**), as do the nudibranchs *Hervia peregrina*, *Flabellina affinis* (**Photos 121**), and *Flabellina lineata* (**Photo 125**) that feed and lay their eggs upon them (Cimino, De Rosa, De Stefano & Sodano, 1980; D'Auria, Minale & Riccio, 1993). In the integument of *Cratena peregrina* (**Photo 124**) there are prenylphenols (**Atlas 163**) (Ciavatta, Trivellone, Villani & Cimino, 1996). The cnidae of this nudibranch have been studied by means of electron

microscopy and other techniques and it would appear that they are used defensively (Martin, 2003; Martin & Walther, 2003).

Two genera of aeolids that feed upon Anthozoa have been found to use chemical defense. *Phestilla melanobrachia* (**Photo 127**) contains alkaloids derived from 6-bromoindole (**Atlas 603**) that also occur in the hard coral upon which it feeds, *Tubastraea coccinea* (Okuda, Klein, Kinnel, Li & Scheuer, 1982). The other genus of interest is *Phyllodesmium* (Rudman, 1981, 1991; Burghardt & Wägele, 2004; Burghardt, Evertson, Johnsen & Wägele, 2005). **Photo 123** shows two specimens of *Phyllodesmium magnum*, a species currently under investigation. These nudibranchs feed upon soft corals (Octocorallia) and appropriate their symbiotic algae for their own use, effectively becoming photosynthetic animals. In this case the cnidae are quite small and they are not used defensively. The nudibranchs are very well camouflaged on the corals, and look very much like their zooids. The cerata, which resemble tentacles, autotomize readily. *Phyllodesmium longicirrum* eats *Sarcophyton trocheliophorum* and uses its terpenoids in defense (Coll, Bowden, Tapiolas, Willis, Djura, Streamer & Trott, 1985). *Phyllodesmium guamensis* has been found to contain a diterpene, 11β-acetoxypukalide (**Atlas 362**) derived from *Sinularia maxima* and *S. polydactyla* (Slattery, Avila, Starmer & Paul, 1998). The metabolite, which is deterrent to fish, is most abundant in the cerata.

Chapter XII

Macroevolution and Macroeconomics

Our historical narrative provides an example of what has been called an "adaptive radiation." From an initial ancestor the opisthobranchs and pulmonates have diversified and have come to flourish in a wide variety of habitats and to exploit a broad range of food items. The idea that chemical defense has been the driving force behind the evolution of these animals helps us to make sense out of their adaptive radiation. The opisthobranchs have been expanding and diversifying for a long period of time, and those processes have been furthered by the diversification of their metabolite-rich food items. They have tended to track their food along taxonomic or genealogical lines, but this is only a tendency. We have seen several examples of opisthobranchs shifting to taxonomically quite distant food items that have the same kind of secondary metabolites. We have also seen the capacity for *de novo* biosynthesis emancipating opisthobranchs and pulmonates from dependency upon their original food items. In many cases the metabolites thus synthesized are polypropionates.

When predator and prey evolve together there may be "coevolution," meaning that the two exercise a reciprocal influence upon one another at the populational level. Supposedly, for example, the presence of dorid nudibranchs in the environment of sponges would bring about selection pressures causing the sponges to evolve the kind of defenses that are effective against the nudibranchs, and the nudibranchs would evolve means of coping with such defenses, such as the ability to detoxify particular secondary metabolites. However, the defenses of the sponges, whether mechanical, chemical, or whatever, would be deployed against a variety of grazers, including fish, and generalists as well as the specialist nudibranchs. The sponges, which derive their sustenence by filtering small particles out of the water, have occupied a wide range of habitats. The opisthobranchs have undergone their adaptive radiation as components of the economy of nature as a whole. The phenomena reviewed in the present work suggest ways in which we might explain the global patterns of biotic diversity from a causal point of view, thereby contributing to the emerging science of bioeconomics (Landa & Ghiselin, 1999).

Adam Smith (1776), in *The Wealth of Nations*, showed how the division of labor increases the output of an enterprise by such advantages as not wasting time by switching from one task to another. Darwin (1859), in *The Origin of Species*, pointed out that such specialization increases the total amount of life that the natural economy can support. There is, however, an important difference between the cooperative division of labor that occurs within productive units, and the competitive division of labor that occurs between them (Ghiselin, 1974, 1978). An organism or a firm is optimized so as to maximize the productivity of the organism or the firm as a whole, but this is not the case for species or other *laissez-faire* economic units, whether natural or political, in which the components receive no return on doing that what is optimal for the whole. The division of labor does indeed tend to contribute to the prosperity of such competitive economies, but only as an incidental result of the advantages realized by the competitors of which the economies are composed. Likewise Charles Babbage suggested that the division of labor makes it possible to put a wider variety of talents to work. It does have that effect, but again, that is not the reason why labor is divided in modern industrial society. As Adam Smith emphasized, bakers are not trying to benefit anybody but themselves when they act so as to put bread on everybody's table. Specialists and their employers simply take advantage of opportunities that are created as a result of labor being divided. Adam Smith made the very important point that the division of labor is limited by the extent of the market. In order to function most effectively as a specialist, there must be enough buyers of one's product or one's service to keep one working full time. For that reason the variety of occupations is greater in larger cities, as can be seen from the fact that medical specialists are concentrated there. Large universities likewise have a greater variety of courses and a greater diversity of professors than small colleges do. For much the same reason, smaller islands, in comparison to larger ones, have a lesser diversity of species, accompanied by a lesser diversity of ways of making a living.

One point that Adam Smith and subsequent economists neglected is that there are both advantages and disadvantages to dividing labor. Of course it has been widely recognized that when there is a recession, some professions are particularly apt to suffer from unemployment. But there are distinct advantages to combining labor. Opisthobranchs are simultaneous hermaphrodites. The advantage is thought to lie in an ability to reproduce at low effective population densities. One disadvantage is that an hermaphrodite must pay the "fixed costs" that are necessary for reproducing as a male as well as those that are necessary for reproducing as a female. Whether natural selection favors hermaphroditism or separate sexes depends upon the particular conditions under which the organisms exist. Among the situations in which combining labor has its advantages are those in which putting different things together has a synergistic effect, the outcome being more than that which is realized when the parts operate separately (see Corning, 2005).

The adaptive radiation of the opisthobranchs may be considered an entrepreneurial affair. They have specialized because that has allowed them to take advantage of economic opportunities. They have not specialized in order to avoid competition with one another, any more than professors of zoology teach that subject in order to avoid competition with their colleagues who teach history or Greek. It is not difficult to see how this division of labor among opisthobranchs has been accomplished. They have been able to exploit quite a variety of food items and of chemicals that they can use in their own defense. Their adaptive radiation has often involved innovations — new technologies, if you will. The evolution of the capacity for *de novo* synthesis of secondary metabolites originally obtained from food is the most remarkable example, but by no means the only one. That capacity had its precursors in the ability to transform secondary metabolites. Much as there are places, such as Silicon Valley, where technological innovation flourishes, there are also places in the natural economy where something analogous goes on. And much as the greatest amount of spe-

cialization within academic enterprises is to be found in the largest universities, there are places in the world where a vast natural economy is exceedingly rich in specialists though it contains a substantial number of generalists.

Opisthobranchs are most diverse — not only in the number of their species and higher taxa, but also in the manner in which they make a living — in the Indo-West-Pacific, especially in the area around New Guinea and the Philippines. The same is true of many other groups of animals and plants, including the ones upon which opisthobranchs feed. The richness of the biota correlates with relatively high and stable temperatures as well as the extent of the habitat. The high and stable temperatures are the result of the global pattern of water movement. In the Eastern Pacific cool water moves from high latitudes toward the equator and then moves westward, accumulating heat from the sun on the way. After crossing the Pacific, water is deflected and moves poleward in both hemispheres, giving up heat as it goes, and recirculates.

The Caribbean fauna is considerably less diverse than that of the Indo-West-Pacific. It is generally considered to have become depauperate because of extinction, though it contains an unusually high number of gorgonians. The Mediterranean is likewise depauperate for historical reasons. That it is still economically undersaturated is clear from the phenomenon of "Lessepsian invasions" from the Red Sea via the Suez Canal. The migrants include opisthobranchs with patterns of chemical defense that facilitate their entering the Mediterranean and getting established there (Mollo, Gavagnin, Carbone, Castelluccio, Pozone, Roussis, Templado, Ghiselin & Cimino, 2008).

Although the global pattern is by no means a simple one, there is a clear tendency for biotic diversity within many taxa to decline as one gets away from the faunal peaks, especially with increasing latitude. That the over-all pattern for opisthobranchs is a real phenomenon can be seen from a comparison of the fauna of various geographically comparable areas, broken down into taxonomic subunits. Here we reproduce figures from an earlier study (Ghiselin, 1992). Madang, on the northern coast of New Guinea has the longest faunal list, with Guam slightly behind, the isolated but well-studied archipelago of Hawaii, much less, and Sagami Bay in Japan, again well studied, a distant fourth:

	Madang	Guam	Hawaii	Sagami Bay
(Total list)	536	410	244	184
Cephalaspidea	70	93	47	5
Anaspidea	9	8	11	13
Sacoglossa	61	86	34	18
Shelled	11	17	8	2
Shell-less	50	69	26	16
Thecosomata	4	11	(0)	(0)
Notaspidea	8	7	12	3
Nudibranchia	382	205	140	145
Doridacea	250	155	91	75
Suctoria	14	2	3	6
Nonsuctoria	56	23	14	17
Cryptobranchia	141	96	60	42
Porostomata	30	24	14	10
Dendronotacea	24	7	6	19
Arminacea	9	3	3	20
Aeolodiacea	99	40	40	31

(It is worth mentioning that Gosliner and his collaborators now have a list of 640 species from Anilao, in the Philippines.)

Trends in biotic diversity have also been extensively documented for the fossil record. The approach has generally involved compilations from the paleontological literature and there are serious methodological problems with it. For one thing, species are even harder to identify in fossil specimens than they are in living ones. Furthermore, although we have a good, objective definition for the species category, there are none for higher levels, such as the genus and the family. Therefore a family of gastropods is not equivalent to a family of bivalves or crustaceans. Nonetheless there does seem to be a general trend toward increasing diversity throughout the fossil record, in spite of numerous mass extinctions. At least some groups have tended to expand more than others in the long run. Bivalves provide a good example. There has been a considerable amount of discussion as to what features of the bivalves might account for their long-term success, and for the decline of the brachiopods with their bivalved shells. The results have seemed inconclusive. When a group expands it may do so because it is occupying previously unexploited niches or it may mean that it is displacing competitors. Their relatively poor fossil record notwithstanding, there is reason to think that opisthobranchs are an expanding group of animals. Viewing them in their present condition provides us with an opportunity to assess the reasons for their long-term, as well as their present, prosperity.

In addition to trends in the number of taxa, there are trends in the properties of the organisms, including the amount of defense, both mechanical and chemical. Comparable trends, corresponding with the conditions of existence, can be found in all groups of organisms, both marine and terrestrial. The size of the body and the size of societies are both good examples. Reproductive trends include the number of eggs laid in a bird's clutch and the amount of sex (in the sense of genetical recombination). From a bioeconomic perspective these patterns can all be treated as variations upon a single theme (Ghiselin, 1974).

As mentioned in a previous chapter, trends in the amount of chemical defense parallel those in species diversity. For prosobranch gastropods, Vermeij (1978, 1987) has documented a closely-comparable trend in mechanical defense: passing westward across the Pacific there is an increasing proportion of snails with heavy shells. He has argued that there has been an "arms-race" through geological time between snails and the crabs that feed upon them. Having joined the ranks of bioeconomists Vermeij (2004) has attempted to explain the trends through time and across space in terms of the amount of energy. The standing crop of animals and plants must have something to do with the energy supply, but what is cause and what is effect? It seems unlikely that the absolute amount of energy flowing into the habitat will explain things in a straightforward and simple manner. Rather it is a matter of how the energy gets utilized by the organisms. And if one looks at Vermeij's raw data, one sees that although it is true that the proportion of heavily-armored gastropods increases, this is because the heavily-armored species are added to the fauna, and not because they replace the others. The absolute numbers of more lightly armored species remain about the same. Areas of low diversity generally contain many organisms of common species, whereas those of high diversity generally contain fewer organisms per species because the species tend to be rare. Armament is expensive and in some parts of the habitat the costs may not be worth the expenditure. An animal may remain under cover, perhaps living beneath rocks and in cavities. It may have nocturnal habits. Well-defended gastropods are able to venture forth into the open to feed. Nudibranchs of the family Phyllidiidae are able to survive and feed upon sponges out in the open in broad daylight because of their highly-developed chemical defense (Brunckhorst, 1991). That allows them to exploit a food supply that would otherwise be left largely alone by spongivores. Among prosobranchs the life history of cowries provides an instructive example (Ire & Iwata, 2005). The young ones have weak shells and remain under cover. Upon reaching a certain size, they stop growing and develop much stronger shells. They are then in a much better position

to venture out, forage, and reproduce. **Photo 1** shows the cowrie *Cyphoma gibbosum* in an exposed position on a gorgonian. These are adults. The juveniles occur at the base of the branches.

Although it may be tempting, it is misleading to conceptualize an adaptive radiation as if it were merely a subdivision of pre-existing niches into increasingly small compartments with an inelastic supply of resources available to their occupants. For similar reasons it is misleading to conceptualize an "arms race" between predators and prey as if it were a "zero-sum game." The adaptive radiation may have an entrepreneurial character, with the radiating group taking advantage of "business opportunities" and expanding by virtue of utilizing previously underexploited resources.

Comparative physiologists have often observed that tropical marine animals tend to live closer to their tolerance limits than do their temperate relatives. That translates into the tropical ones running a greater risk of mortality through overheating or drying out. From a bioeconomic point of view the next question to ask is not what causes them to die under such circumstances but rather whether, as a consequence of taking such risks, they are able to occupy an appropriate habitat and turn a profit in the form of reproductive success. In the tropics one often finds marine animals, such as both anomuran and brachyuran crabs, that have been "displaced" upward toward higher levels, so that they become more like terrestrial ones. Such animals are "pushing" against their economic limits, as well as their physiological ones.

Among the various components of an animal's habitat are its predators. Tropical opisthobranchs seem to be living closer to the limits of their capacity to deal with them as well. In such a struggle for existence the use of defensive metabolites might well give an animal competitive edge. In the literature on chemical ecology the relationships between predation and geographical patterns of chemical defense have often discussed (see Hay, 1996; Hay & Steinberg, 1992; Cronin, Paul, Hay & Fenical, 1997; Becerro, Turon, Uriz & Paul, 2003). There has been a lot of talk about differences in the amount of "predation pressure" from one place to another. This is supposed to translate into predators preventing unprotected animals from flourishing in the tropics. This perspective confuses cause and effect. Instead of thinking in terms of the predators having the negative effect of preventing the growth of undefended prey populations, what we really need to consider is the positive effect of defensive adaptations in allowing the prey populations to expand. Selection is not a negative force that prevents something from happening, but a mechanism whereby resources are allocated one way or another.

The Indo-West-Pacific diversity peak occurs in a region that is climatically stable and the biota is relatively saturated economically. If one looks at higher latitudes one generally finds a lower diversity of opisthobranchs, and this trend correlates with seasonality of the environments. The difference between tropical and higher-latitude dorid nudibranchs have lately been addressed by Andersen, Desjardine, and Woods (2006). They draw attention to the fact that many of the dorids of the northern coasts of the Eastern Pacific are able to biosynthesize their own defensive metabolites. And yet such animals as *Cadlina luteomarginata* feed upon quite a variety of sponges and may utilize dietary metabolites as well as those that they make themselves. On the other hand tropical nudibranchs seem not to do that, but to derive their metabolites from food. They argue (pp. 297-298) that there is a high diversity of food items in the tropics and that, therefore, "tropical nudibranchs have ready access in their diets to sequesterable defensive chemicals and consequently they should have little need to make repugnant chemicals via *de novo* biosynthesis." On the other hand, the food items in higher latitudes are less diverse and less rich in metabolites. This seems not to be quite what is going on. The tropical nudibranchs live in a less seasonal environment. Therefore, they are specialized for a way of life in which the larvae home in on a particular source of food and metabolites that other animals are ill-adapted to exploit. They spend a consid-

erable amount of time as larvae in the plankton finding the appropriate habitat and are able to do so by virtue of the very fact that the environment is not so seasonal. The higher-latitude nudibranchs, on the other hand, live in a seasonal environment, where there is little time available for homing in on the food supply with its metabolites. Their way of life involves scrambling for food and turning it into sperm and eggs as soon as possible. Biosynthesis of defensive metabolites is expensive, but it pays in terms of less time and effort expended seeking them out.

The Antarctic seems to present an exception to the commonly-accepted pattern of chemical defense being largely a tropical phenomenon (McClintock & Baker, 1998; Amsler, Iken, McClintock & Baker, 2001). The Antarctic plankton is very "productive," but only in the sense of producing a high standing crop of plants and animals, so that it supports a food chain with crustaceans, fish, and whales. The growing season is, however, short, and the organisms scramble for food. The benthos seems to have quite different conditions of existence. It is dominated by slow-growing and sessile or slow-moving animals. As in the deep sea, from which the biota is partly derived, food is scarce. The animals do not experience the kind of seasonal influx of food that would allow them to grow and reproduce rapidly and thereby outpace the growth of predator populations. *Bathydoris*, as suggested above, is an animal that has expanded its range of food items so as to eat more than just sponges. That allows it to feed under the prevailing conditions of scarcity. Evolving the capacity for biosynthesis of defensive metabolites means that it can defend itself without being dependent upon a particular kind of food item.

Being common at higher latitudes, *de novo* synthesis has sometimes been described as a "cold water" phenomenon. Especially if one pays attention only to some dorid nudibranchs, one might get the impression that temperature *per se* has something to do with it. But *de novo* synthesis occurs in *Dendrodoris* and *Doriopsilla* of warmer waters as well. Furthermore, it is quite common in warm-water representatives of other opisthobranch taxa. We need only consider the family Tethyidae, which evidently became emancipated from dependency upon tropical cnidarians. The family, which is largely tropical and subtropical, gave rise to some lineages of colder waters, including the Oregonian *Melibe leonina*.

All that we can infer from the gradients is that, for some reason, it seems advantageous to invest relatively more in chemical defense under the conditions that occur in tropical economies. That the latitudinal gradients are not necessarily due to the availability of metabolites in the food supply is indicated by the occurrence of such gradients in animals that biosynthesize these *de novo*. The gradient discussed above (in Chapter 4) for onchidal in various species of the marine pulmonate genus *Onchidella* makes that quite clear. The tendency of sponges to contain lesser amounts of secondary metabolites in higher latitudes and more at lower ones has also been known for many years (Green, 1977). Such a tendency might favor *de novo* synthesis on the part of nudibranchs that feed upon them.

We must also reject as unfounded the frequently-expressed notion that a high level of species diversity in a given area is the result of a high rate of speciation. To be sure, there is good reason to believe that coexisting and closely related species of opisthobranchs often have different food organisms. But this does not mean that speciation events are either the cause or the effect of the phenomenon. What really matters is not the origin of species, but rather their ability to get established and maintain themselves in the natural economy. Likewise in the political economy, it may be easy to set up a business, but what really counts is whether the business, once established, is able to return a net profit.

From what we know of speciation processes in marine animals generally, it is clear that speciation is going on all the time. The supposed lack of sufficient geographical isolation to allow for species formation reflects nothing more than lack of imagination. New species are coming into the

biota as cryptic ones that soon evolve minor differences in behavior, physiology and way of life. Those differences may be sufficient to prevent ecological exclusion, which is the analogue of being driven out of business, thereby allowing a particular species to maintain itself once it has become established. Among those differences are a diversity of defensive metabolites. We may recollect the cryptic species of bryozoans, each with its own symbiotic bacteria, producing different alkaloids. Also mentioned was the fact that in marine sponges there are numerous cryptic species and these show differences in their autecology. Further diversification is contingent upon additional speciation, along with innovations in the way of making a living. Again, speciation rate does not seem to constrain the process very much. The rate of innovation is another matter.

The animals, plants, and microorganisms that synthesize secondary metabolites and use them defensively can evolve new metabolites by modifying the biosynthetic pathways. Often this is done in a straightforward manner, for example, by adding another acetate unit to a polyketide or another isoprenoid unit to a terpene. Such terminal addition leads to longer chains, and it also allows for a greater variety of structurally-modified metabolites, such as those that have undergone rearrangement. Comparative studies documenting such changes are available for algae (Amico, 1995) and sponges (Van Soest, Fusetani & Andersen, 1998). Another way of acquiring new metabolites is by entering into symbiotic relationships, something that is well documented for sponges. The acquisition of any new metabolite is an example of an evolutionary innovation. It is like the introduction of a new drug into the pharmaceutical industry. Evolutionary innovation also may be involved in the origin of the capacity to produce some kind of change in a molecule: for example the esterification of a terpenoid by a dorid nudibranch or the methylation of polyketides by fungi. Innovation has also occurred in acquisition of the capacity for biotransformation. And the abilities to form concentrations of metabolites in repugnatorial glands, to store them there, and to release them at the appropriate time and place, represent a whole series of innovations. The opisthobranchs have been remarkably innovative in their acquisition and use of secondary metabolites. But they are also remarkable for some other innovations that affect their prosperity. Two examples of "organelle symbiosis" have been mentioned. One is the defensive use of stinging capsules appropriated by aeolid nudibranchs from the cnidarians upon which they feed. Another is sacoglossans turning themselves into something like motile leaves by farming chloroplasts within their own bodies. In the latter case a new kind of enterprise has opened up previously untilled fields for cultivation.

The opisthobranchs have radiated adaptively on a vast scale. We might well compare their adaptive radiation with that of other taxa. The phenomenon has been examined in groups that have diversified on isolated archipelagos. A particularly well studied case is "Darwin's finches" of the Galápagos (Grant & Grant, 2008). A more spectacular case is the honeycreeper finches of Hawaii (Amadon, 1947). There are also well-studied examples of adaptive radiations that have taken place in freshwater lakes, most notably the cichlid fishes of Africa (Fryer & Iles, 1972; Meyer, 1993; Salzburger & Meyer, 2004). Some common features of such radiations are noteworthy. In the first place, the groups that have diversified so much have filled something of an economic vacuum in doing so. Secondly, although island endemics quite generally have become considerably modified in adaptation to local circumstances, only a small minority of taxa within a biota have actually undergone adaptive radiations. Thus, although the Galápagos birds include flightless cormorants, only one group, the Geospizidae, has actually undergone an adaptive radiation. Thirdly, diversification seems to take place rather slowly at first, suggesting that it takes a while for the organisms to evolve whatever it takes to diversify extensively. And fourth, the lineages that do radiate have what has been called "diversification potential" by Grant and Grant (2008). The Galápagos finches owe this potential in part to their behavioral flexibility, and in part to the ability of the beak to be modified so as to function in different ways, thereby exploiting different kinds of food items.

The Hawaiian honeycreepers have likewise diversified in bill shape and feeding habits. Likewise, the functional morphology of the African cichlids allows them to occupy a remarkable range of feeding niches (Greenwood, 1981; Liem, 1973).

The aforementioned vertebrate radiations have taken place within, geologically speaking, a relatively short period of time. The adaptive radiation of opisthobranchs is more of a long-term affair, but it fits more or less the same pattern. The pulmonates are a peculiar case, for there has been only a modest diversification of them in the sea, whereas the Basommatophora have become major components of the freshwater biota and Stylommatophora a very successful group of terrestrial organisms. The marine pulmonates, with their use of chemical defense, live very much like opisthobranchs. The diversification potential of opisthobranchs depends mainly upon their ability to utilize the same resource both as nutriment and for provision of defensive metabolites. On the other hand, *de novo* synthesis allows them to emancipate themselves from their original source of metabolites.

The fossil record of opisthobranchs is not complete enough to tell us whether there was an initial period of slow evolution, but a comparison of groups that have radiated extensively with their more primitive relatives definitely suggests that this is what happened. The cephalaspideans include just a few lineages that are rich in species, and these are the most highly modified anatomically. Among sacoglossans there are a few shelled forms, some of them remarkable for such peculiarities as bivalved shells. But most of the diversification has taken place in lineages that have abandoned the shell and switched to different food items and new kinds of chemical defense. The notaspideans would seem to have undergone a minor radiation that foreshadows the more extensive one in the various lineages of nudibranchs.

The Galapagos finches have undergone the first adaptive radiation to be recognized as such. That happened soon after Darwin returned from his epoch-making journey around the world. In the second edition of his *Journal of Researches* Darwin (1845:380) only alluded to their evolutionary significance: “Seeing this gradation and diversity of structure in one small, intimately related group of birds, one might really fancy that from an original paucity of birds in this archipelago, one species has been taken and modified for different ends.” The finches have been intensively studied and have yielded a great deal of insight into the underlying evolutionary processes. We have mentioned just a few of the other adaptive radiations that have been studied by biologists. Some of these are striking, indeed spectacular, examples of nature’s creativity.

The radiation of opisthobranchs is not just spectacular; it is downright astounding. The gastropod radula might be compared to the avian beak, getting pressed into service in exploiting a variety of food items. The opisthobranchs, however, are adept not just in feeding, but in what they do with the food and with the metabolites therein contained, once the food has been ingested. The ability to accumulate, modify, and deploy secondary metabolites has been the basis for their prosperity. But opisthobranchs have been adept, not just at finding ways to put such metabolites to use, but at evolving the capacity to produce what previously they merely consumed. And this has been accomplished in two different, and equally remarkable, ways. The first has been to evolve a biosynthetic pathway that starts with the same pool of primary metabolites and yields essentially the same secondary metabolites that occurred in the food supply, perhaps duplicating the intermediate steps. The second has been to evolve a biosynthetic pathway that incorporates precursors such as propionate, acetate and mevalonate, and yields metabolites that resemble those of other organisms both in structure and function, but may differ fundamentally from these in their composition as well as their genesis. The narrative account that we have provided for the adaptive radiation of opisthobranchs is about the evolution of biosynthetic capacity — like the origin of life itself.

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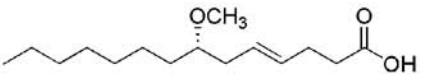
APPENDIX I

An Atlas of Metabolite Structure

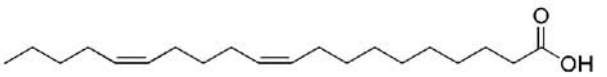
For list of organisms as sources of the metabolies and literature references see Appendix II

Polyacetates

- Fatty acids

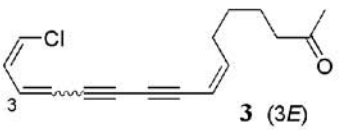


1 7*S*-methoxy-4-tetradecaenoic acid

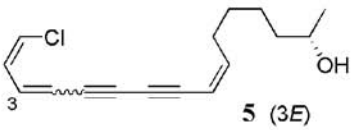


2 10,15-eicosadienoic acid

- Polyacetylenes

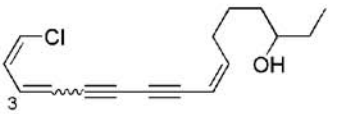


3 (3*E*)
4 (3*Z*)

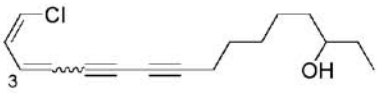


5 (3*E*)
6 (3*Z*)

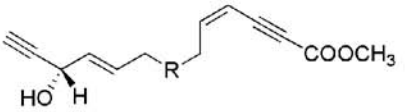
7 R_1=H, R_2=OH
8 R_1=OH, R_2=H



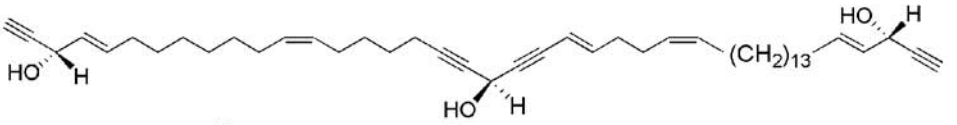
9 (3*E*)
10 (3*Z*)



11 (3*E*), R = H

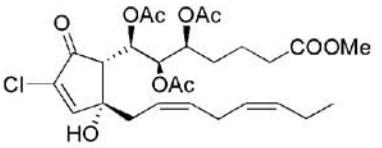


12 R=$C_{31}H_{56}$ petroformyne-A

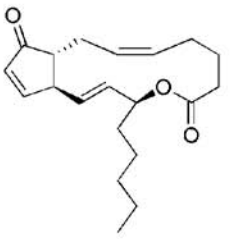


13 petroformyne-1

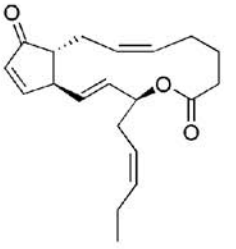
- Prostaglandin lactones



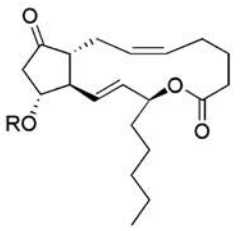
14 punaglandin-1



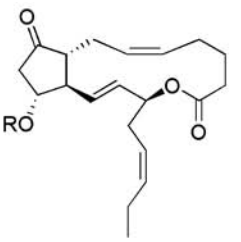
15 PGA_2-1,15-lactone



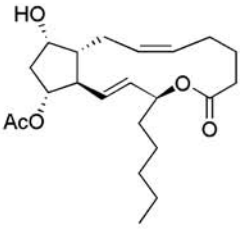
16 PGA_3-1,15-lactone



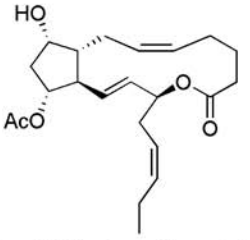
17 PGE_2- 1,15-lactone R= H
18 PGE_2-1,15-lactone-11-acetate R = Ac



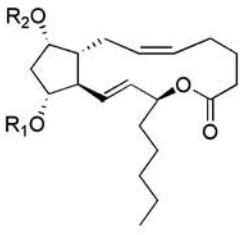
19 PGE_3- 1,15-lactone R= H
20 PGE_3-1,15-lactone-11-acetate R = Ac



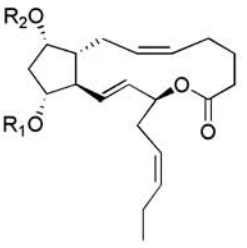
21 $PGF_{2\alpha}$-1,15-lactone-11-acetate



22 $PGF_{3\alpha}$-1,15-lactone-11-acetate



23 $PGF_{2\alpha}$ -1,15-lactone
9- or 11-fatty acid esters

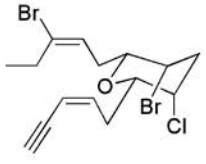


24 $PGF_{3\alpha}$ -1,15-lactone
9- or 11-fatty acid esters

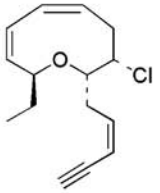
R_1 = H, R_2 = fatty acyl
or
R_1 = fatty acyl, R_2 = H

R_1 or R_2 = palmitic + arachidonic + eicosapenta-*cis*-5,8,11,17-enoic + *cis*-docosahexa-4,7,10,13,16,19-enoic acyl

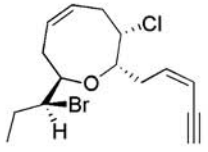
- Cyclic acetogenins



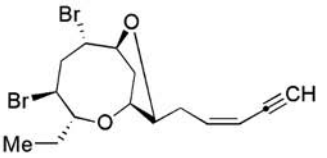
25 dactylyne



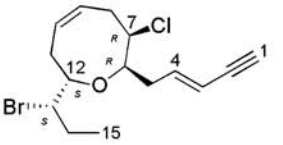
26 brasilenyne



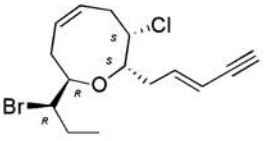
27 *cis*-dihydrorhodophytin



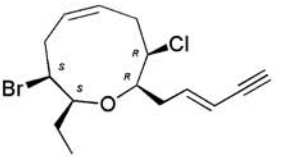
28 (3*Z*)-bromofucin



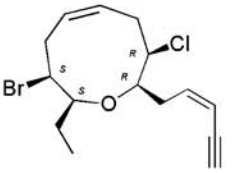
29 (-)-6*R*,7*R*,3*E*-pinnatifidenyne



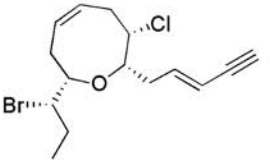
30 (+)-12*R*,13*R*,3*E*-pinnatifidenyne



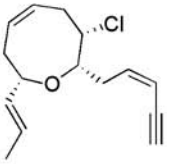
31 (+)-6*R*,7*R*,3*E*-obtusenyne



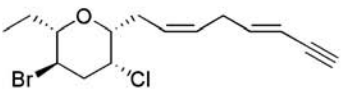
32 (+)-6*R*,7*R*,3*Z*-obtusenyne



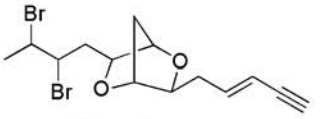
33 (+)-3-*E*-pinnatifidenyne



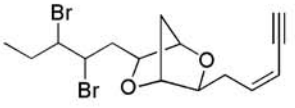
34 (+)-laurenyne



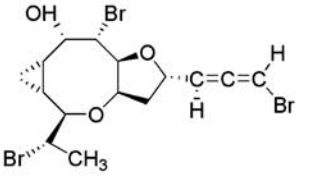
35 srilankenyne



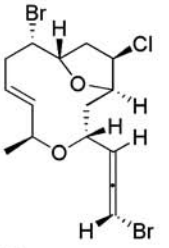
36 ocellenyne a



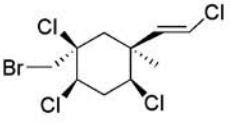
37 ocellenyne b



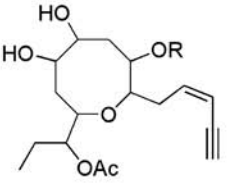
38 aplyparvunin



39 dactylallene

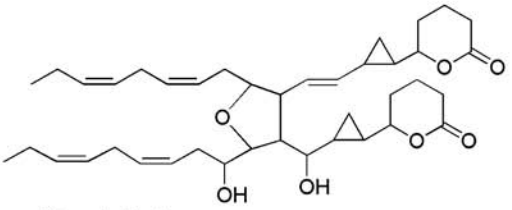


40 violacene

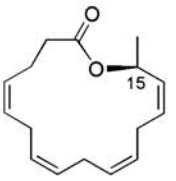


41 doliculol A R = H
42 doliculol B R = Ac

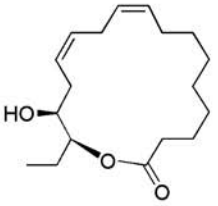
- Macrocyclic fatty acid lactones



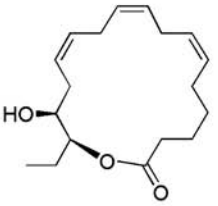
43 aplydilactone



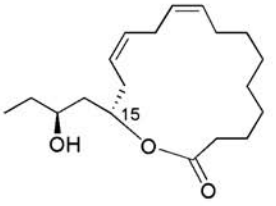
44 aplyolide-A



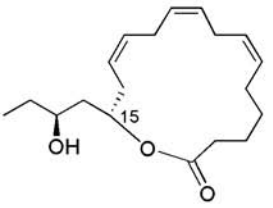
45 aplyolide-B



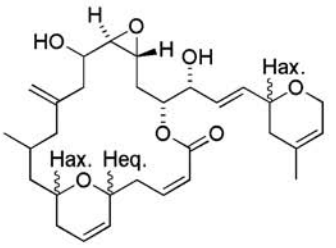
46 aplyolide-C



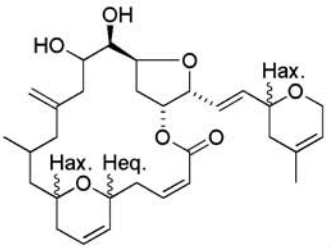
47 aplyolide-D



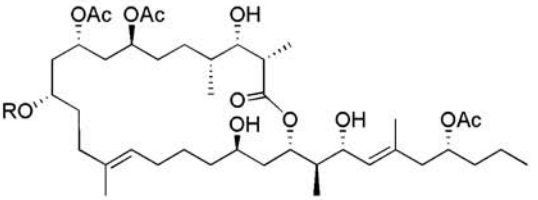
48 aplyolide-E



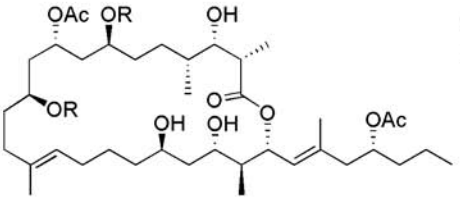
49 laulimalide



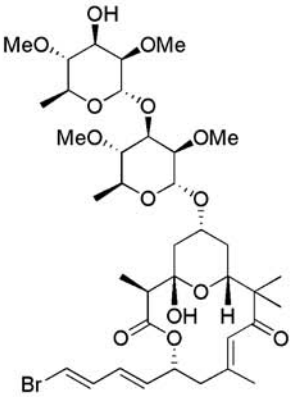
50 isolaulimalide



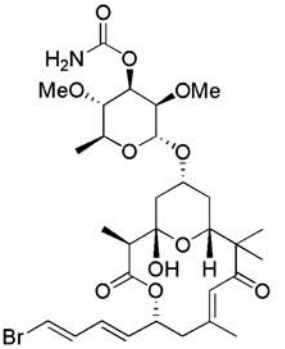
51 dolabelide-A R = Ac
52 dolabelide-B R = H



53 dolabelide-C R = Ac
54 dolabelide-D R = H

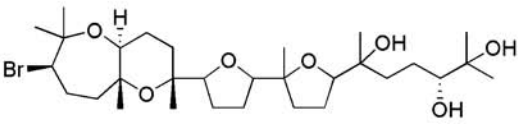


55 auriside-A

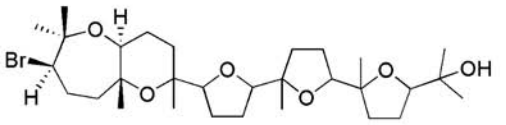


56 auriside-B

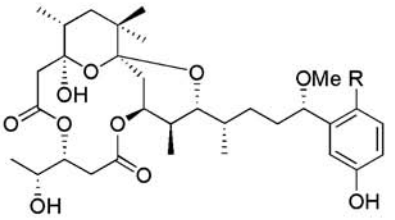
Polyethers



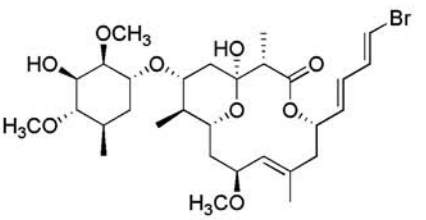
57 aurilol



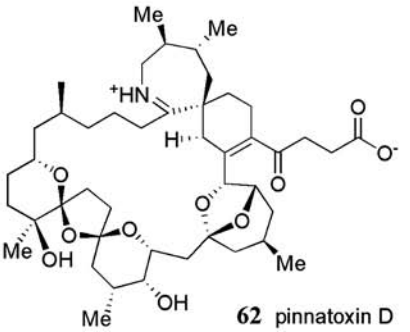
58 enshuol



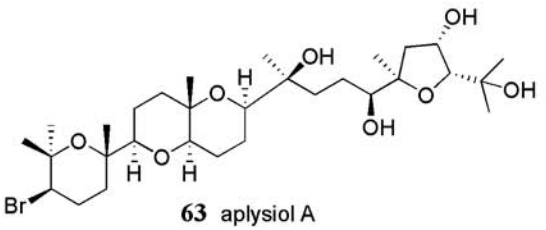
R = **59** Br aplysiatoxin
60 H debromo aplysiatoxin



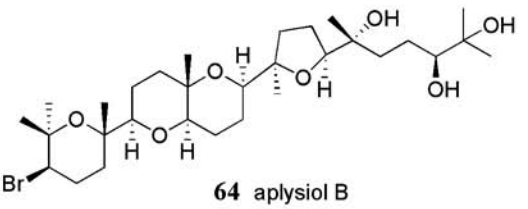
61 dolastatin 19



62 pinnatoxin D

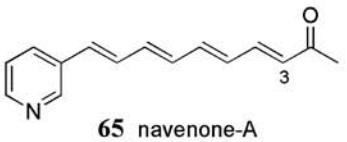


63 aplysiol A

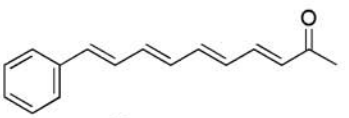


64 aplysiol B

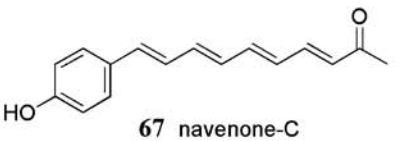
Aromatic Polyketides



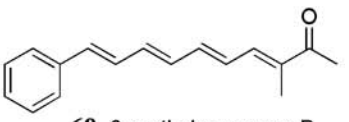
65 navenone-A



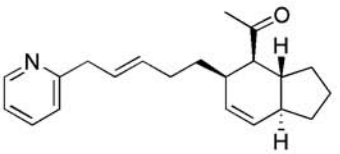
66 navenone-B



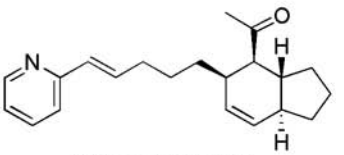
67 navenone-C



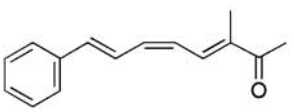
68 3-methylnavenone-B



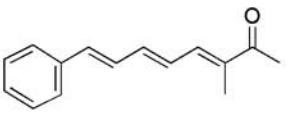
69 pulo'upone



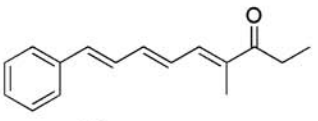
70 isopulo'upone



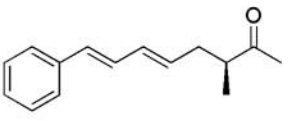
71 lignarenone-A



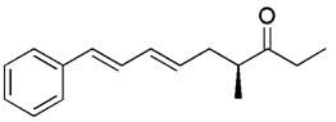
72 lignarenone-B



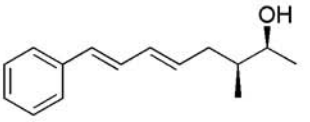
73 lignarenone-C



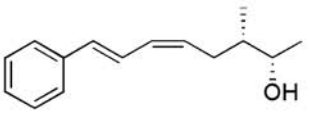
74 dihydrolignarenone-B



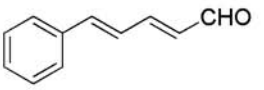
75 dihydrolignarenone-C



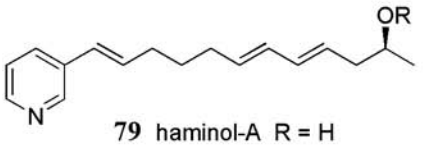
76 dihydrolignarenol-B



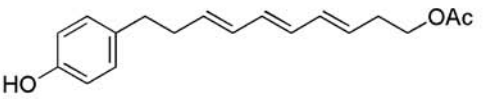
77 dihydrolignarenol-A



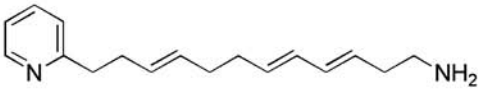
78 ω-arylpenta-2,4-dienal



79 haminol-A R = H
80 haminol-B R = Ac

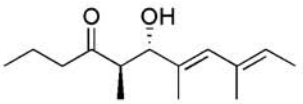


81

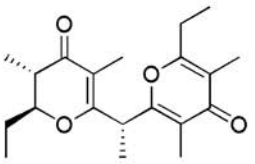


82 naloamine

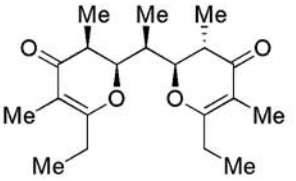
Polypropionates



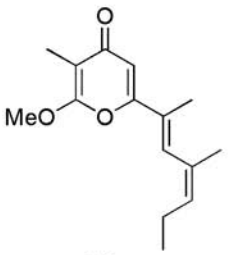
83 pteroenone



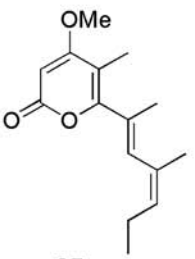
84 vallartanone-B



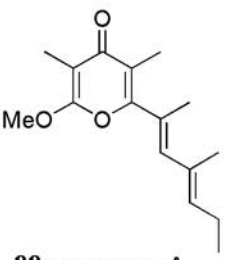
85 (-)-membrenone-C



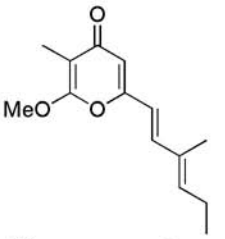
86



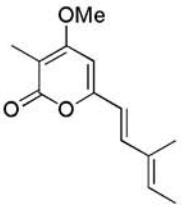
87



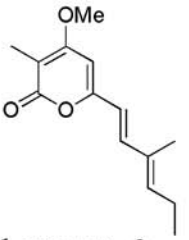
88 cyercene - A



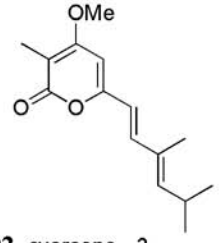
89 cyercene - B



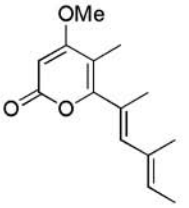
90 cyercene - 1



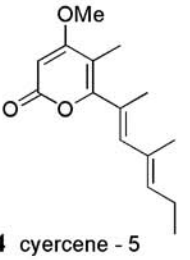
91 cyercene - 2



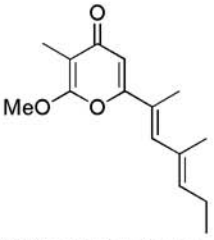
92 cyercene - 3



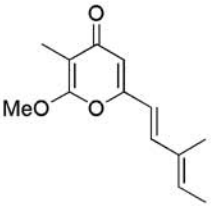
93 cyercene - 4



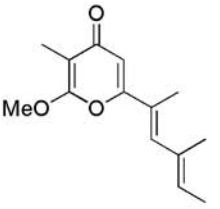
94 cyercene - 5



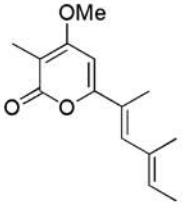
95 7-methylcyercene - B



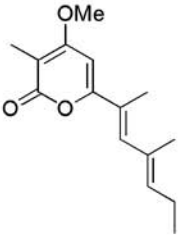
96 12-norcyercene - B



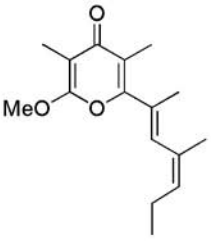
97 7-methyl-12-norcyercene - B



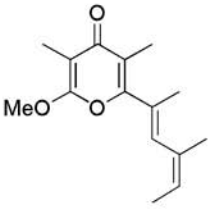
98 7-methyl-cyercene - 1



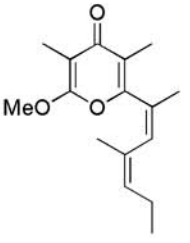
99 7-methyl-cyercene - 2



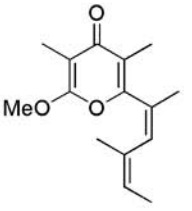
100 placidene - A



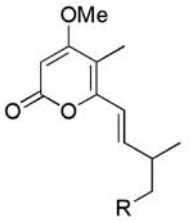
101 placidene - B



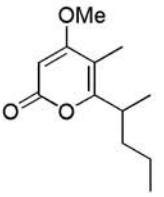
102 isoplacidene - A



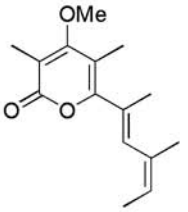
103 isoplacidene - B



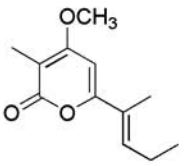
104 R = CH_3
105 R = CH_2CH_3



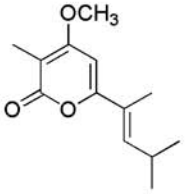
106



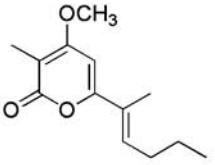
107



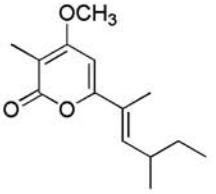
108 aplysiopsene A



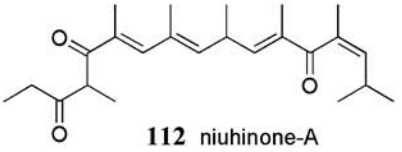
109 aplysiopsene B



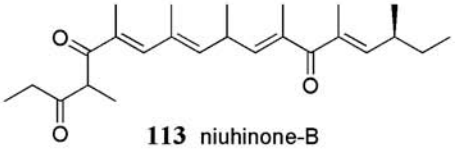
110 aplysiopsene C



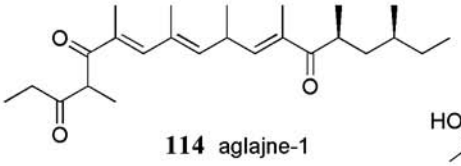
111 aplysiopsene D



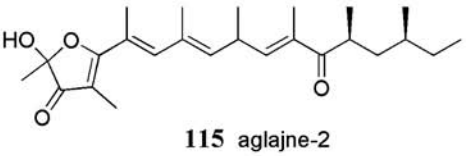
112 niuhinone-A



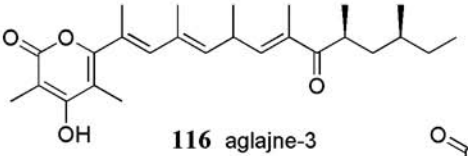
113 niuhinone-B



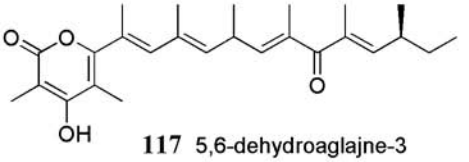
114 aglajne-1



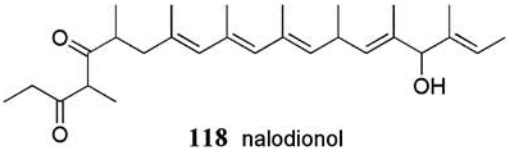
115 aglajne-2



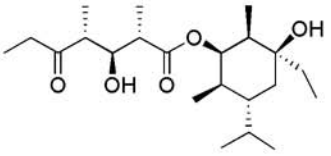
116 aglajne-3



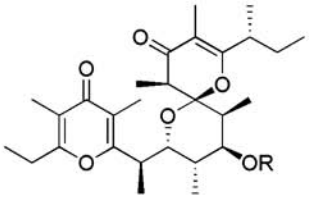
117 5,6-dehydroaglajne-3

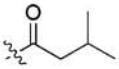


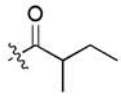
118 nalodionol

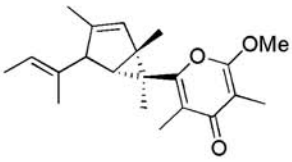


119 dolabriferol

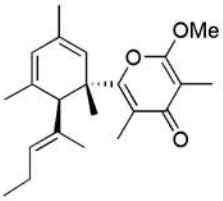


120 auripyrone A R = 

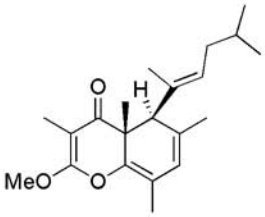
121 auripyrone B R = 



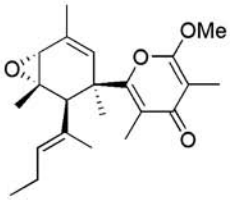
122 15-nor-photodeoxytridachione



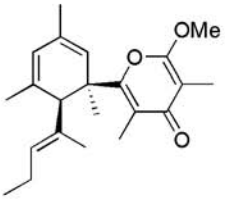
123 *iso*-9,10-deoxytridachione



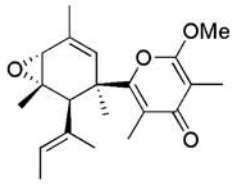
124 tridachiahydropyrone



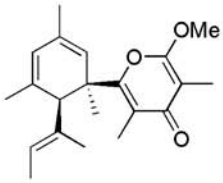
125 tridachione



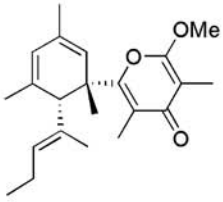
126 9,10-deoxytridachione



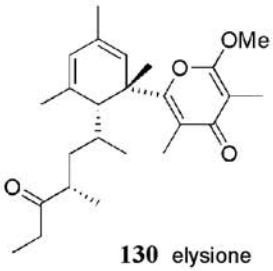
127 15-nor-tridachione



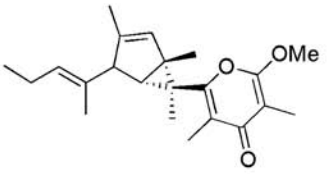
128 15-nor-9,10-deoxytridachione



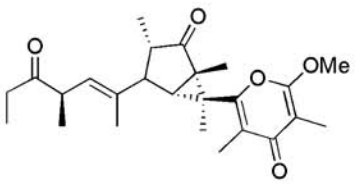
129 *ent*-9,10-deoxytridachione



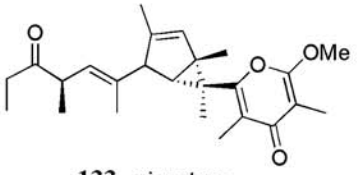
130 elysione



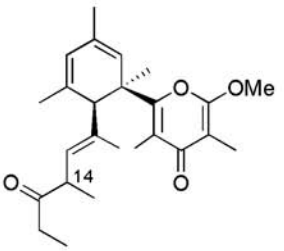
131 photodeoxytridachione



132 crispatone

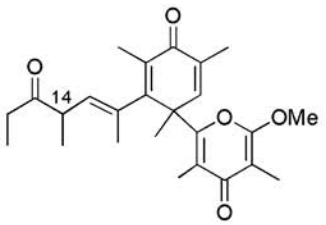


133 crispatene



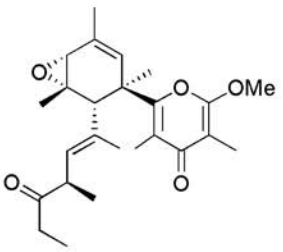
134 (14*R**) tridachiapyrone -A

135 (14*S**) isotridachiapyrone -A

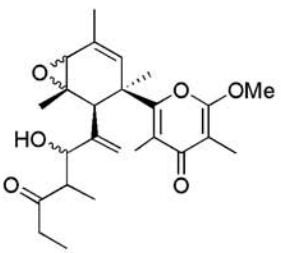


136 (14*R**) tridachiapyrone -B

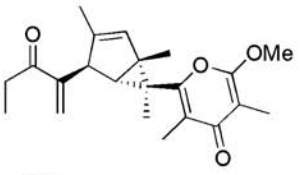
137 (14*S**) isotridachiapyrone -B



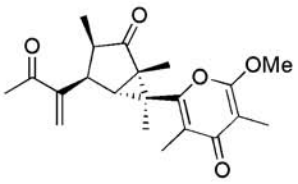
138 tridachiapyrone -C



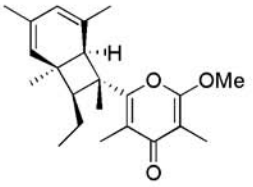
139 tridachiapyrone -D



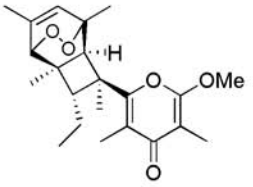
140 tridachiapyrone -E



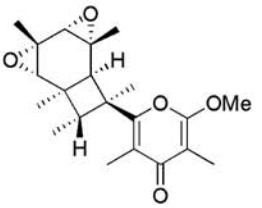
141 tridachiapyrone -F



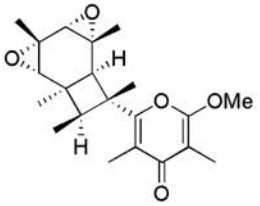
142 ocellapyrone-A



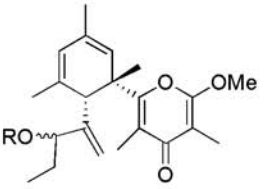
143 ocellapyrone-B



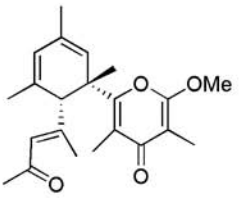
144 elysiapyrone-A



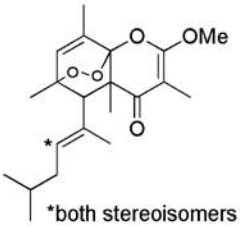
145 elysiapyrone-B



146 R = H, 13 *R* or *S*
147 R = H, 13 *S* or *R*
148 R = OH, 13 *R* or *S*

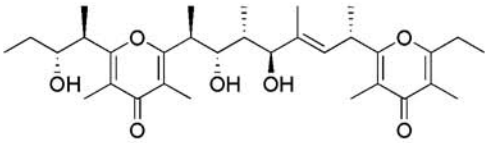


149

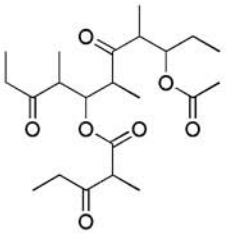


*both stereoisomers

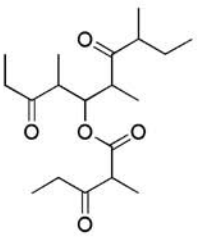
150



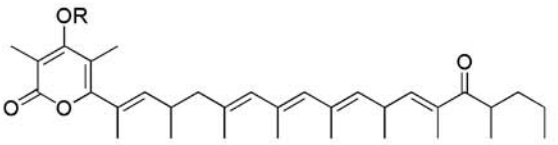
151 ilikonapyrone



152 micromelone A

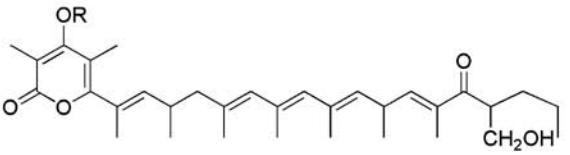


153 micromelone B



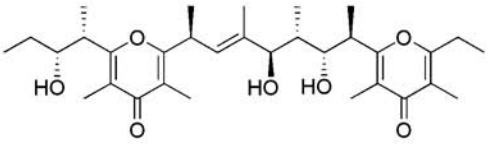
fusaripyrone A

154 R=H
155 R=CH_3

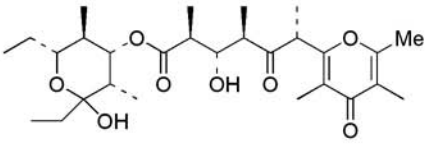


fusaripyrone B

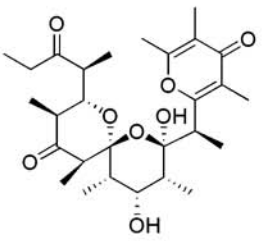
156 R=H
157 R=CH_3



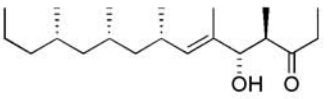
158 peroniatriol I



159 sisserone A

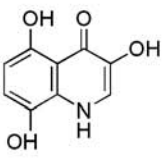


160 siphonarin A

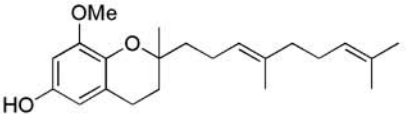


161 siphonarienolone

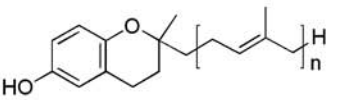
Phenols and Quinones



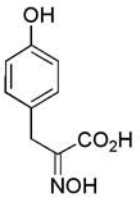
162 uranidine



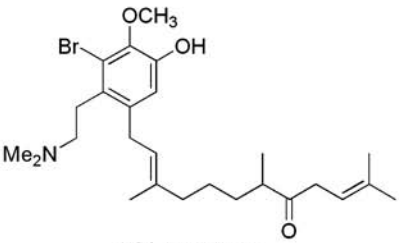
163



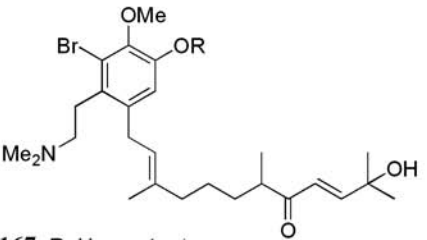
164 n = 1-6 chromanols



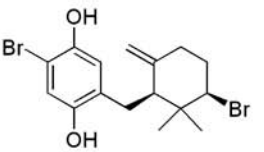
165



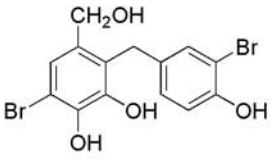
166 aplaminone



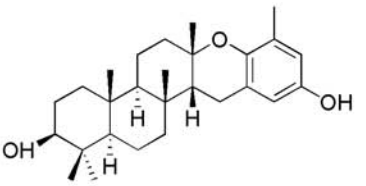
167 R=H neoaplaminone
168 R=SO_3H neoaplaminone sulfate



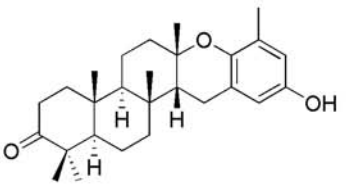
169 cyclocymopol



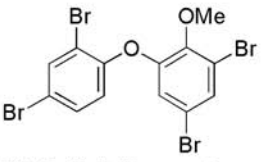
170 avrainvilleol



171 (+)-epitaondiol

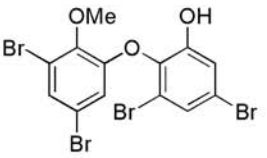


172 3-keto epitaondiol

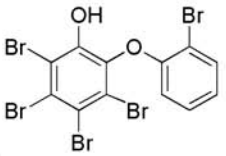


173 2-(2',4'-dibromophenoxy)-4,6-dibromoanisole

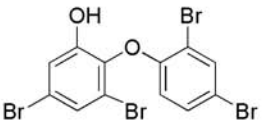
174 4,6-dibromo-2-(2',4'-dibromophenoxy)phenol



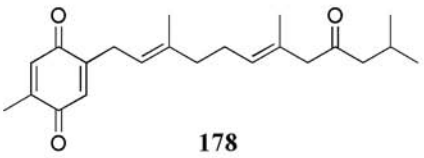
175 3,5-dibromo-2-(3',5'-dibromo-2'-methoxyphenoxy)phenol



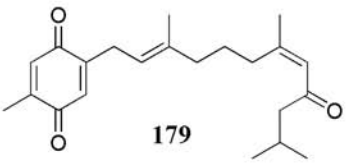
176 2,3,4,5-tetrabromo-6-(2'-bromophenoxy)phenol



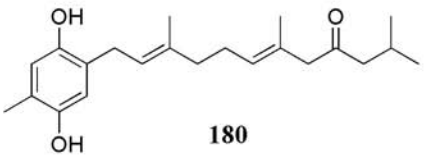
177 3,5-dibromo-2-(2',4'-dibromophenoxy)phenol



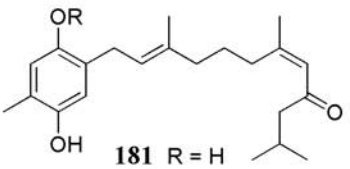
178



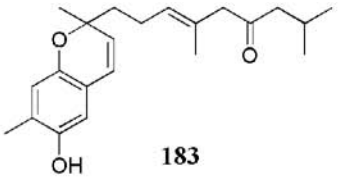
179



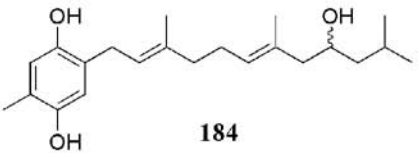
180



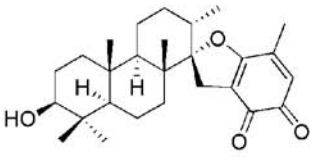
181 R = H
182 R = Ac



183

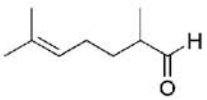


184

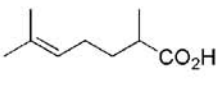


185 (±)-stypoldione

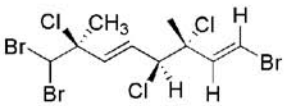
Monoterpenoids



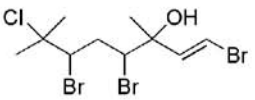
186



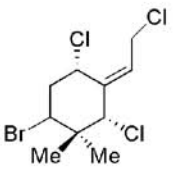
187



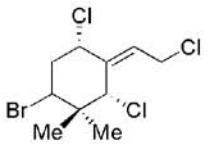
188 (3*R*,4*S*,7*S*)-*trans*,*trans*-3,7-dimethyl-1,8,8-tribromo-3,4,7-trichloro-1,5-octadiene



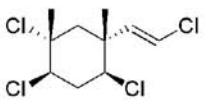
189 3,7-dimethyl-7-cloro-1,4,6-tribromo--3-ol-1*E*-octene



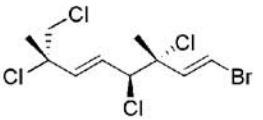
190 apakaochtodene A



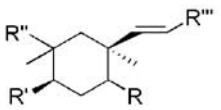
191 apakaochtodene A



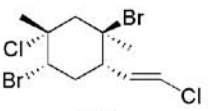
192 aplysiaterpenoid-A



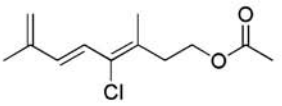
193 aplysiaterpenoid-B



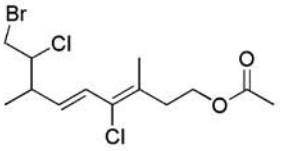
194 R = α-Br R' = Br R'' = β-Cl R''' = Cl
195 R = β-Br R' = Cl R'' = α-Cl R''' = Cl
196 R = β-Br R' = Cl R'' = β-Cl R''' = Br



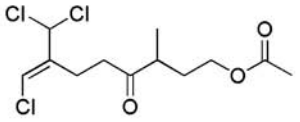
197



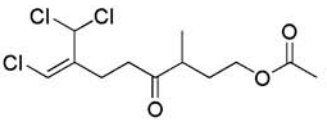
198



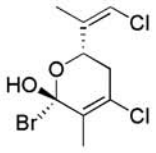
199



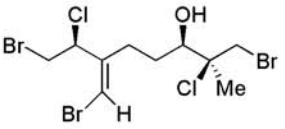
200 (7*E*)-1-acetoxy-8-chloro-7-(dichloromethyl)-3--methyloct-7-en-4-one



201 (7*Z*)-1-acetoxy-8-chloro-7-(dichloromethyl)-3-methyloct-7-en-4-one

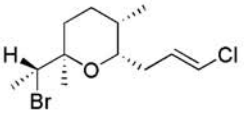


202 costatone

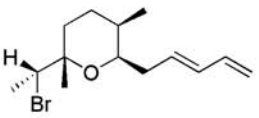


203 kurodainol

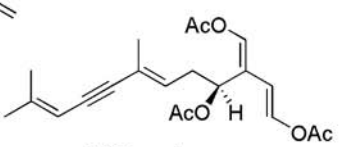
Sesquiterpenoids



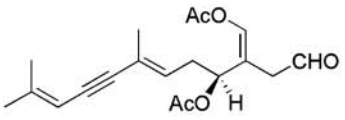
204 kumepaloxane



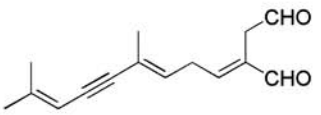
205



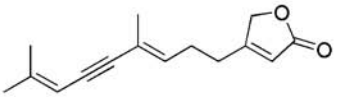
206 caulerpenyne



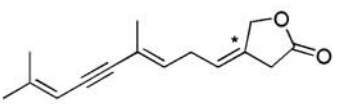
207 oxytoxin -1



208 oxytoxin -2

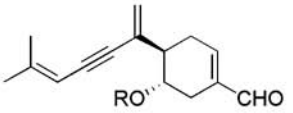


209 ascobullin - A

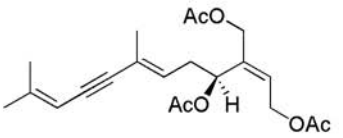


210 ascobullin - B

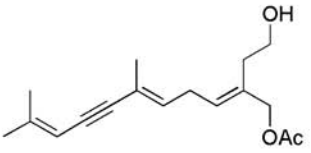
*undetermined stereochemistry



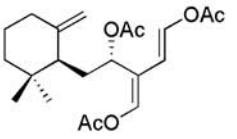
211 R = H volvatellin
212 R = Ac



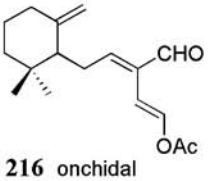
213



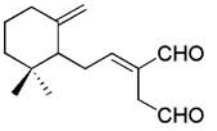
214



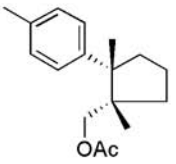
215 crispatenine



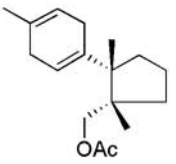
216 onchidal



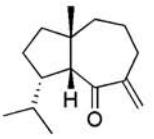
217 ancistrodial



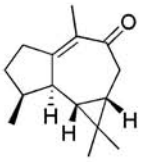
218 tochuinyl acetate



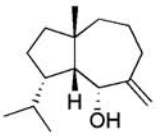
219 dihydrotochuinyl acetate



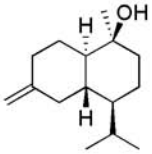
220 millecrone-A



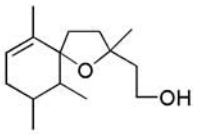
221 millecrone-B



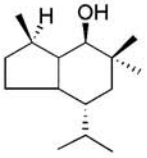
222 millecrol-A



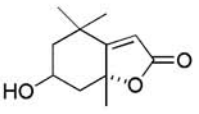
223 millecrol-B



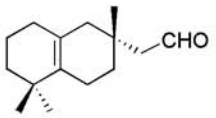
224 dactyloxene-B



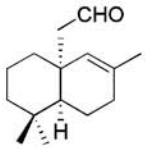
225 (+) brasilenol



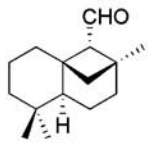
226 (-)-loliolide



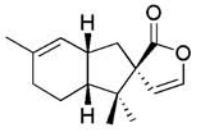
227 nanaimoal



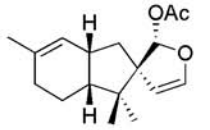
228 isoacanthodoral



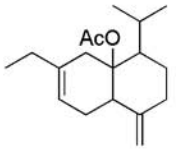
229 acanthodoral



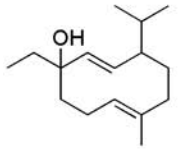
230 dehydroherbadysidolide



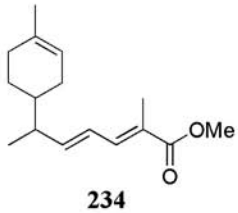
231 spirodysin



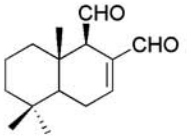
232



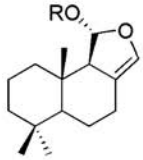
233



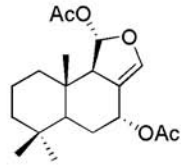
234



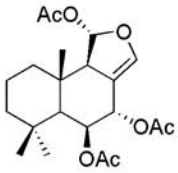
235 polygodial



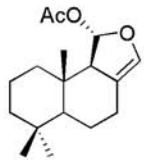
236 R = fatty acyl; drimane esters



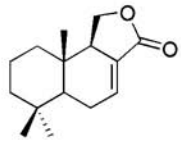
237 olepupuane



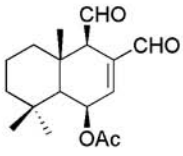
238 6-β-acetoxy-olepupuane



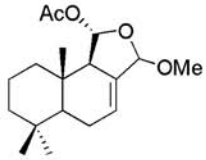
239 7-deacetoxy olepupuane



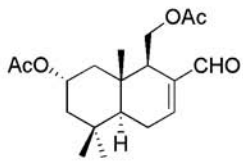
240 cinnamolide



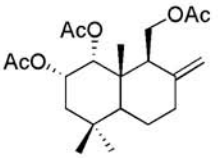
241 6-β-acetoxypolygodial



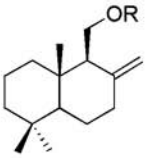
242 drimane methoxyacetal



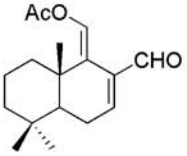
243 hodgsonal



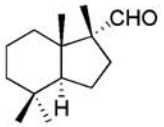
244 1α,2α-diacetoxyalbicanylacetate



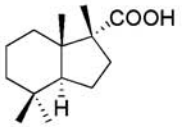
245 R = H (+)- albicanol
246 R = Ac (+)- albicanyl acetate)



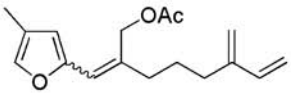
247 pu' ulenal



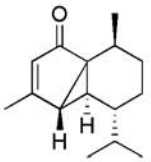
248 austrodoral



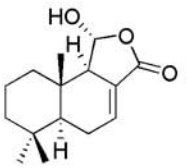
249 austrodoric acid



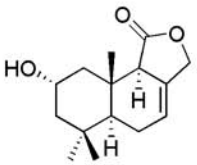
250 algoafuran



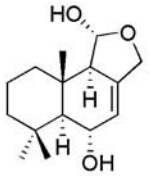
251 cubebenone



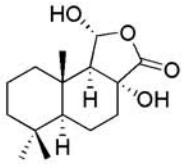
252 dendrocarbin A



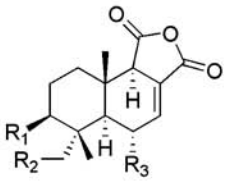
253 dendrocarbin B



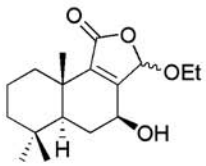
254 dendrocarbin C



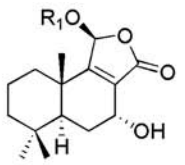
255 dendrocarbin D



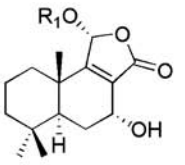
256 dendrocarbin E R_1 = OH, R_2 = R_3 = H
257 dendrocarbin F R_1 = H, R_2 = OH, R_3 = H
258 dendrocarbin G R_1 = R_2 = H, R_3 = OH
259 dendrocarbin H R_1 = R_2 = R_3 = H



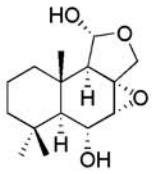
260 dendrocarbin I



261 dendrocarbin J R = Et
262 dendrocarbin K R = H

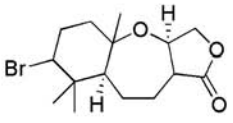


263 dendrocarbin L R = Et
264 dendrocarbin M R = H

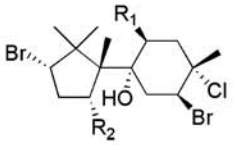


265 dendrocarbin N

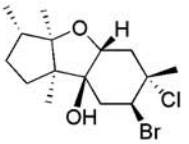
Halogenated sesquiterpenoids



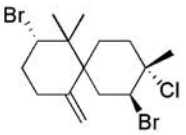
266 aplysistatin



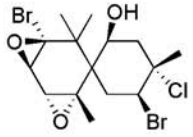
267 algoane R_1 = OAc, R_2 = OH
268 1-deacetoxyalgoane R_1 = H, R_2 = OH
269 1-deacetoxy-8-deoxyalgoane R_1 = R_2 = H



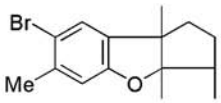
270 ibhayinol



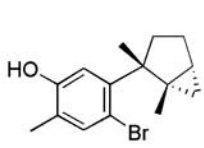
271 nidificene



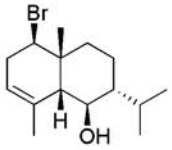
272 prepacifenol epoxide



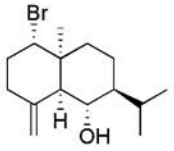
273 aplysin



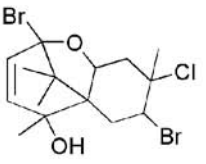
274 cyclolaurenol



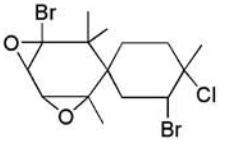
275 lankalapuol A



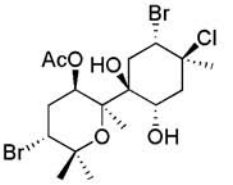
276 lankalapuol B



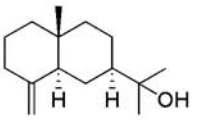
277 pacifenol



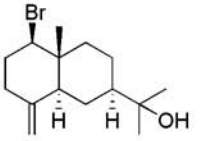
278 dehydroxyprepacifenol epoxide



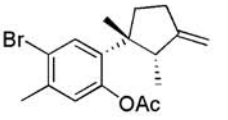
279 dihydroxydeodactol monoacetate



280 brasudol

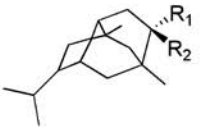


281 isobrasudol

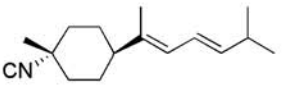


282 allolaurinterolacetate

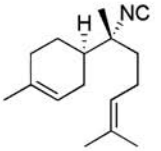
Isocyanosesquiterpenoids



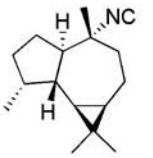
283 R_1 = NC; R_2 =H 9-isocyanopupukeanone
284 R_1 = H; R_2 = NC 9-*epi*-9-isocyanopupukeanone



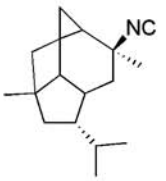
285 isocyanotheonellin



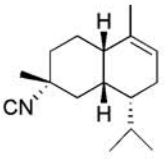
286 7-isocyano-7,8-dihydro-α-bisabolene



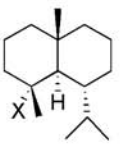
287 axisonitrile-2



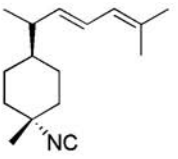
288 2-isocyanoallopupukeanane



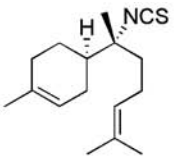
289 4α-isocyano-9-amorphene



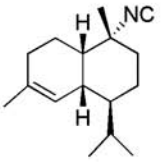
290 X = NC 4α-isocyanogorgon-11-ene
291 X = NCS 4α-isothiocyanatogorgon-11-ene
292 X = NHCHO 4α-formamidogorgon-11-ene)



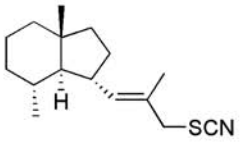
293
3-isocyanobisabolane-8,10-diene



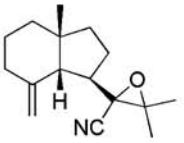
294
7-isothiocyanato-7,8- dihydro-α-bisabolene



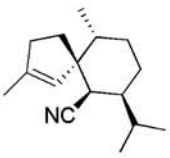
295 10α-isocyano-4-amorphene



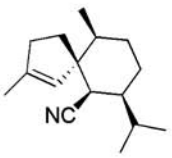
296 cavernothiocyanate



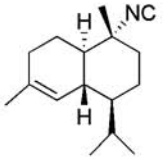
297 cavernoisonitrile



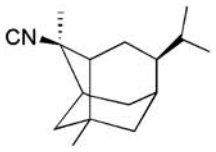
298 axisoisonitrile-3



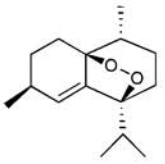
299 10-*epi*-axisoisonitrile-3



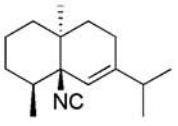
300 10-isocyano-4-cadinene



301 2-isocyanotrachyopsane



302 1,7-epidioxy-5-cadinene



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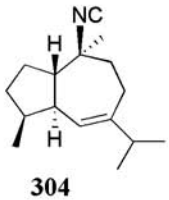

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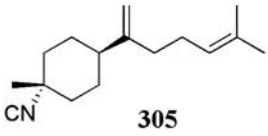

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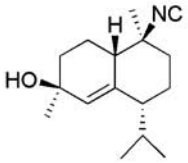

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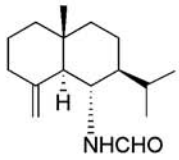
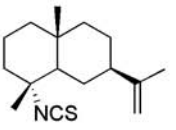


307 acanthene C



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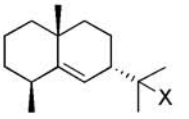
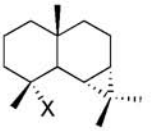
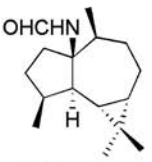


309 X = NCS
310 X = NC



311 X = NHCHO
312 X = NC

313 R_1* = SCN; R_2* = H
314 R_1* = H; R_2* = SCN
*stereochemistry is relative



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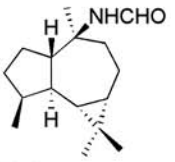
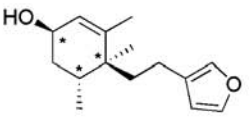
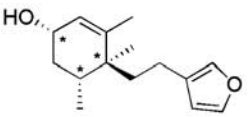


316

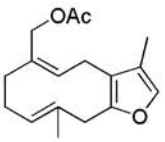
Furanosesquiterpenoids



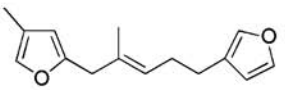


*relative stereochemistry

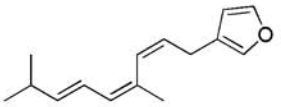
317 pelseneeriol-1 **318** pelseneeriol-2



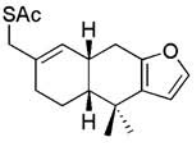
319 julieannafuran



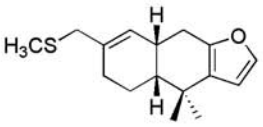
320 dehydrolasiosperman



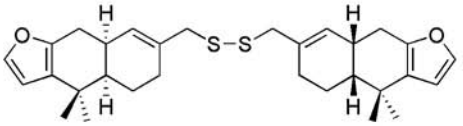
321 *cis*-dehydrodendrolasin



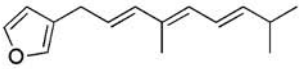
322 thiofurodysinin acetate



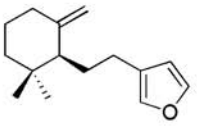
323 methyl thiofurodysinin



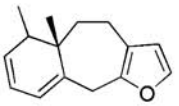
324 dithiofurodysinin



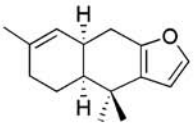
325 dehydrodendrolasin



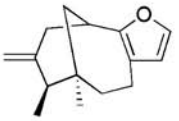
326 dehydropallescensin-2



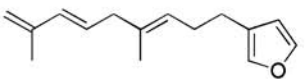
327 agassizin



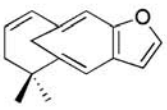
328 (-)-furodysinin



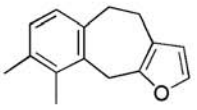
329 isonakafuran 9



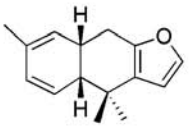
330 isodehydrodendrolasin



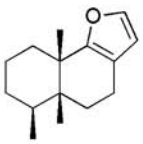
331 spiniferin 1



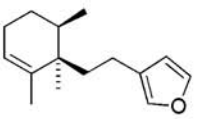
332 spiniferin-2



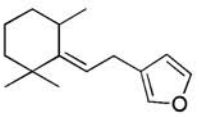
333 dehydrofurodysinin



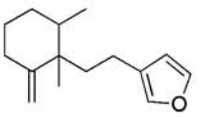
334 microcionin-1



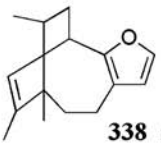
335 microcionin-2



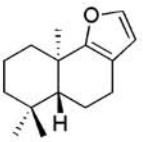
336 microcionin-3



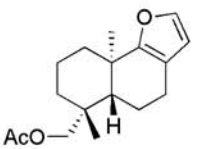
337 microcionin-4



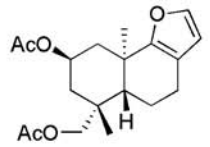
338 nakafuran-8



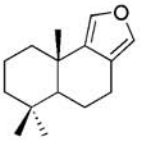
339 (-) *ent*-pallescensin A



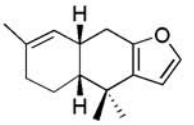
340 15-acetoxy-*ent*-pallescensin A



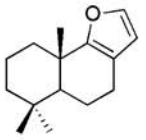
341 2,15-diacetoxy-*ent*-pallescensin A



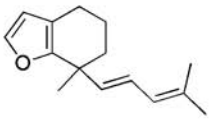
342 euryfuran



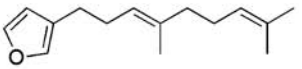
343 (+)- furodysinin



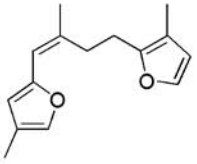
344 (+) pallescensin A



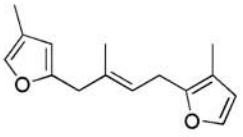
345 laevidiene



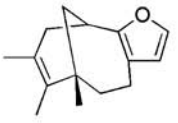
346 dendrolasin



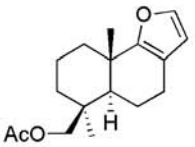
347 tavacfuran



348 longifolin

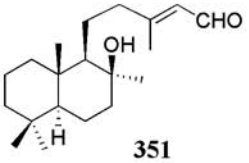


349 nakafuran-9



350 15-acetoxy-pallescensin-A

Diterpenoids



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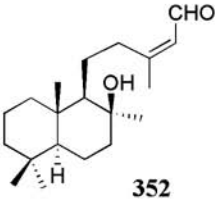

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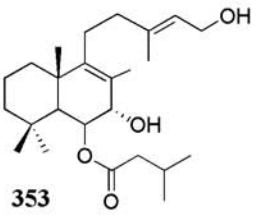

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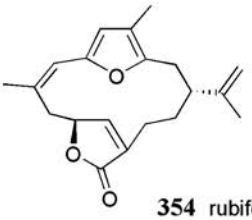
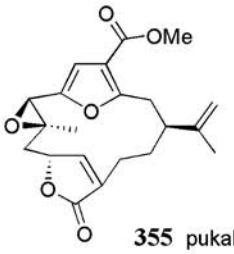
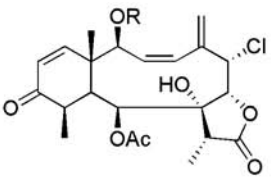


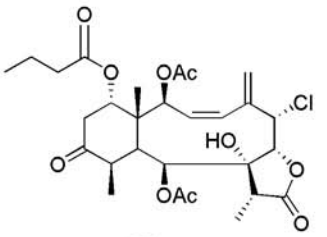
354 rubifolide



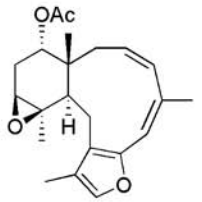
355 pukalide



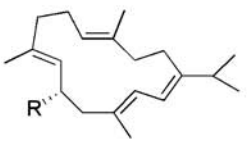
356 ptilosarcenone R = Ac
357 ptilosarcenone butanoate derivative R =



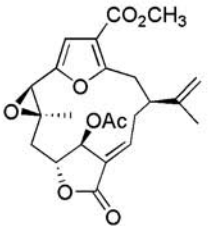
358 ptilosarcone



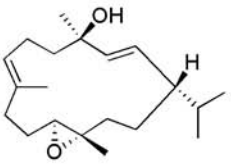
359 (-)-verecynarmin-A



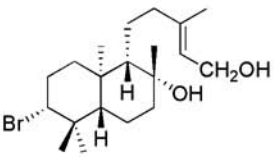
360 (+)-preverecynarmin R = OAc
361 cembrene-C R = H



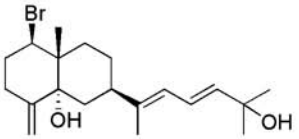
362 11β-acetoxypukalide



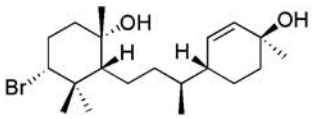
363 trocheliophorol



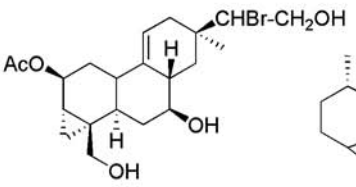
364 aplysin-20



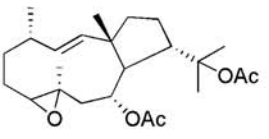
365 aplysiadiol



366 14-bromoobtus-1-ene-3,11-diol



367 parguerol



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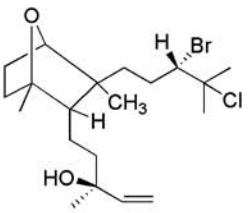
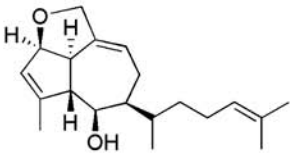
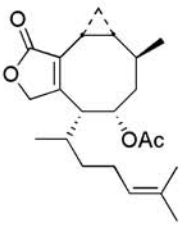


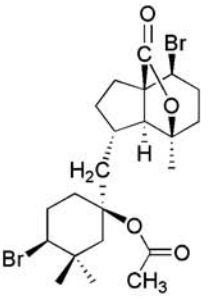
369 dactylomelol



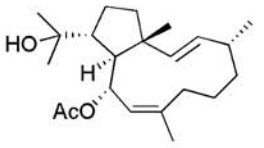
370 dictyol A



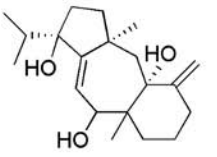
371 acetoxycrenulide



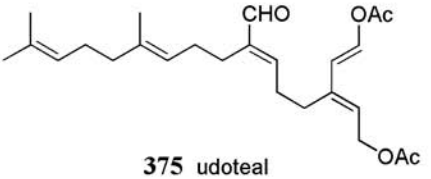
372 angasiol acetate



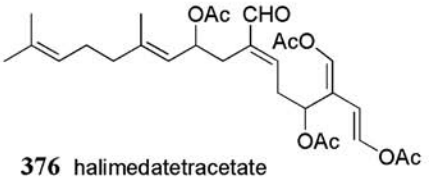
373 10-acetoxy-18-hydroxy-2,7dollabelladiene



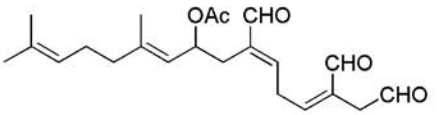
374 dolatriol



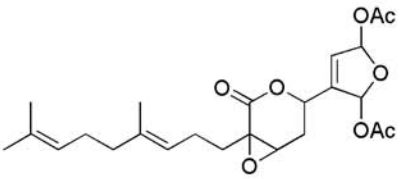
375 udoteal



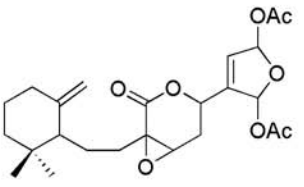
376 halimedatetracetate



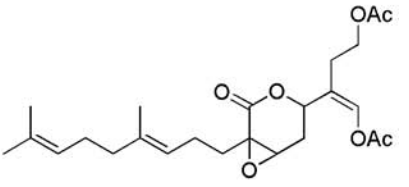
377 halimedatrial



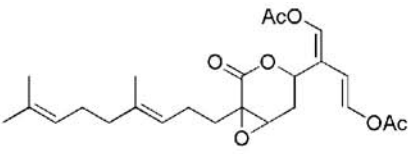
378 thuridillin -A



379 thuridillin -B



380 thuridillin -C



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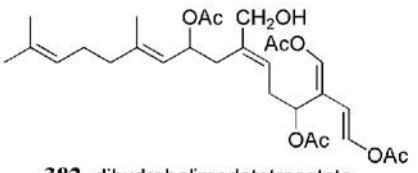
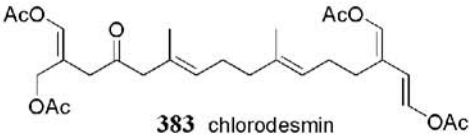
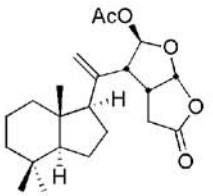


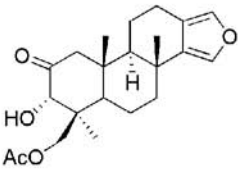
382 dihydrohalimedatetracetate



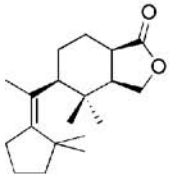
383 chlorodesmin



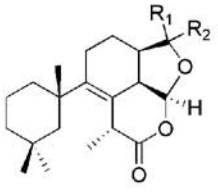
384 norrisolide



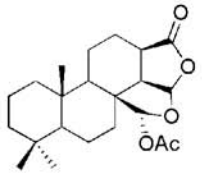
385 spongiadiol-19-acetate



386 glaciolide



387 R_1=R_2 = O; cadlinolide A
388 R_1 = H, R_2 = OAc; tetrahydroaplysulphurin-1
389 R_1 = H, R_2 = OH; cadlinolide B



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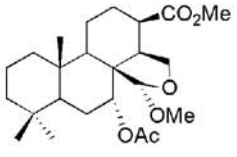
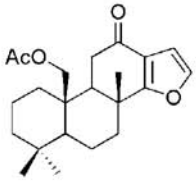
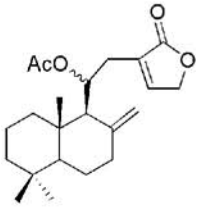


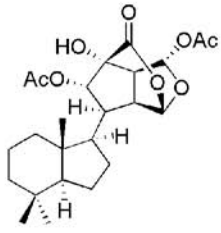
391



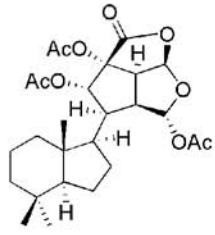
392 20-acetoxy-marginatone



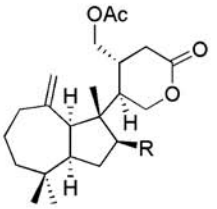
393 lutenolide



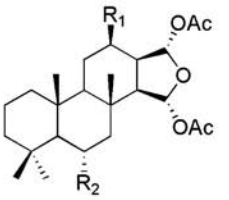
394 chromodorolide A



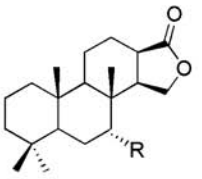
395 chromodorolide B



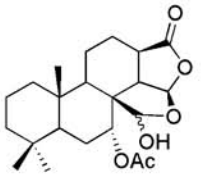
396 R = OAc shahamin K
397 R = H 12-desacetoxyshahamin C



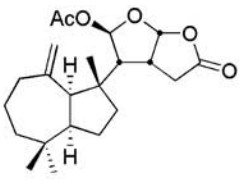
398 R_1 = H; R_2 = OAc
399 R_1 = OAc; R_2 = OAc
400 R_1 = OAc; R_2 = H



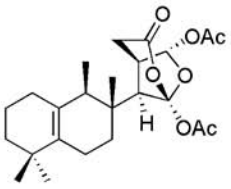
401 R = OAc
402 R = H



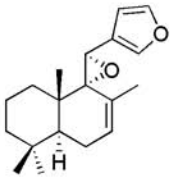
403 aplyroseol 2



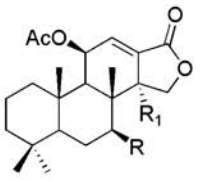
404 dendrillolide A



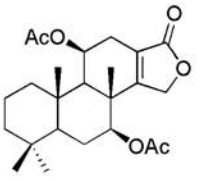
405 shahamin F



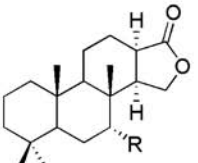
406 ghiselinin



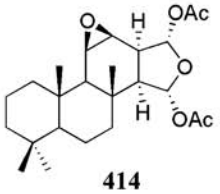
407 R = OAc; R_1 = OH dorisenone A
408 R = H; R_1 = OH dorisenone B
409 R = OAc; R_1 = H dorisenone D



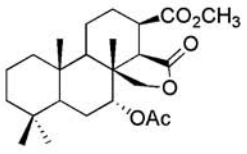
410 dorisenone C



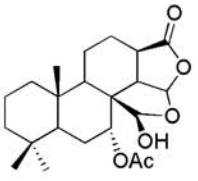
411 R = OH
412 R = H
413 R = OAc



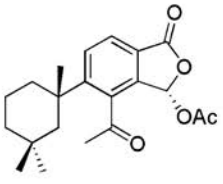
414



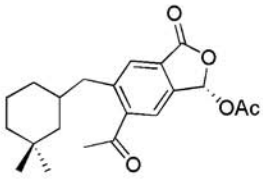
415 7α-acetoxy-dendrillol-3



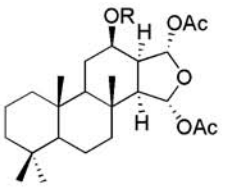
416



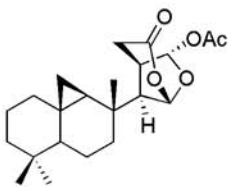
417 macfarlandin A



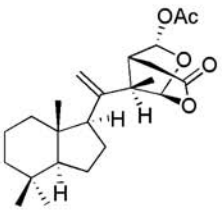
418 luteorosin



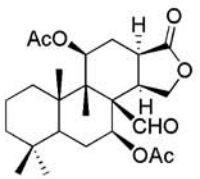
419 R = Ac 12-*epi*-aplysillin
420 R = H 12-*epi*-12-deacetylaplysillin



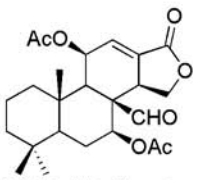
421 polyraphin C



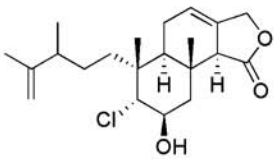
422 chelonaplysin C



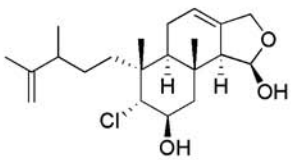
423 7β,11β-diacetoxy-16--oxospongian-17-al



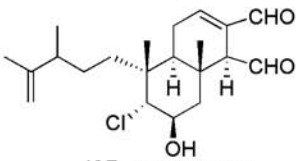
424 7β,11β-diacetoxy-16--oxospong-12-en-17-al



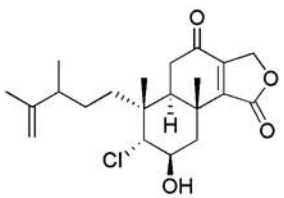
425 hamiltonin A



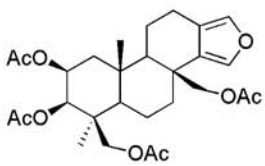
426 hamiltonin B



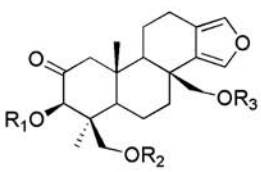
427 hamiltonin C



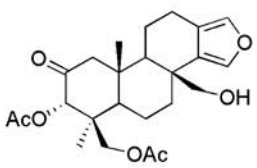
428 hamiltonin D



429



430 R_1 = H; R_2 = R_3 = Ac
431 R_1 = R_2 = Ac; R_3 = H
432 R_1 = Ac; R_2 = R_3 = H
433 R_1 = R_2 = R_3 = Ac
434 R_1 = R_2 = R_3 = H



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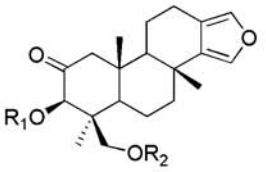
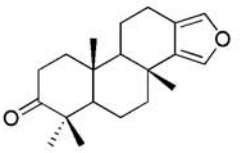


436 R_1 =Ac; R_2 = H
437 R_1 = R_2 = H



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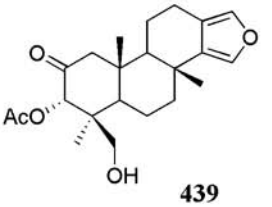

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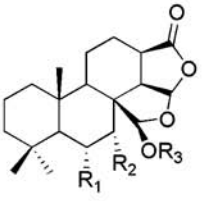
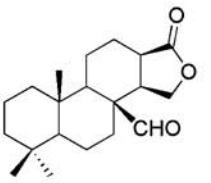
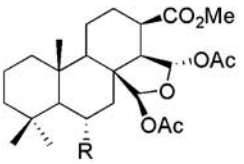


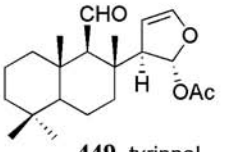
440 R_1 = -OC(O)Pr; R_2 = -H; R_3 = -H
441 R_1 = -Ac; R_2 = -H; R_3 = -H
442 R_1 = -Ac; R_2 = -H; R_3 = -Ac
443 R_1 = -OC(O)Pr; R_2 = -H; R_3 = -Ac
444 R_1 = R_2 = R_3 =-H



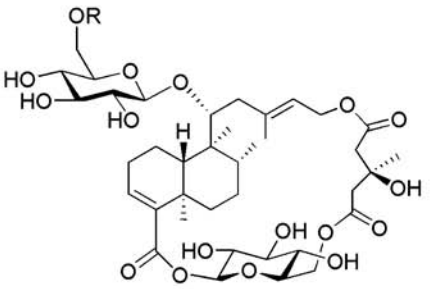
445



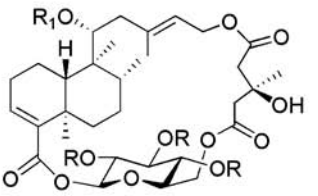
446 R = -OAc
447 R = -OC(O)Pr
448 R = -H



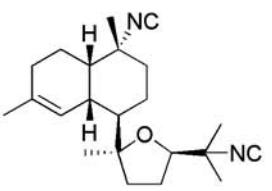
449 tyrinnal



450 syphonoside R = H
451 acetylsyphonoside R = -Ac



452 syphonosideol R = R_1 = H
453 syphonoside esters R = H,
R_1 = $CH_3(CH_2)_{14}CO$-
or $CH_3(CH_2)_{16}CO$-



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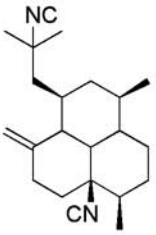

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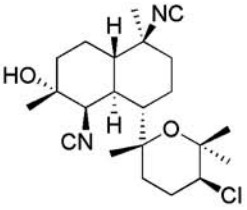

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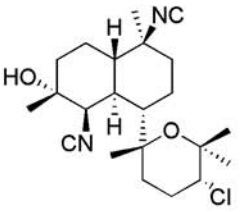

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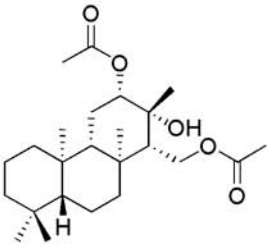

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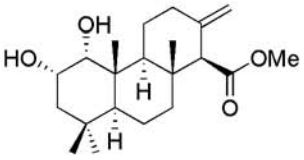
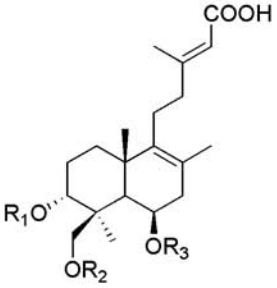
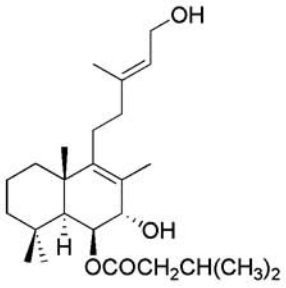


459



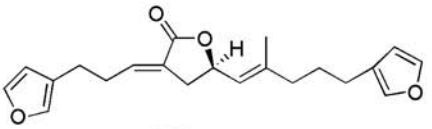
460 $R_1 = R_2 = R_3$
461 $R_1 = COC_4H_9$, $R_2 = R_3 = H$
462 $R_1 = R_3 = COC_4H_9$, $R_2 = H$
463 $R_1 = R_2 = COC_4H_9$, $R_3 = H$



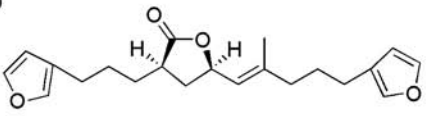
464 6β-isovaleroxylabda-8,13-dien-7α-diol

Sesterterpenoids

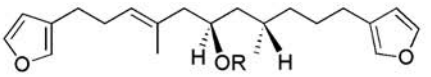
- Degraded furanosesterterpenoids



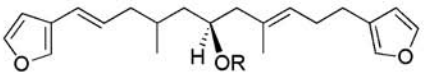
465 nitenin



466 dihydronitenin

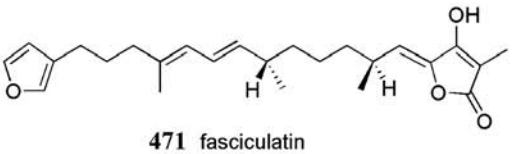


467 R = Ac 11-acetyl-furospongin
468 R = H furospongin

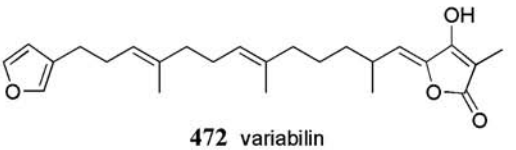


469 R = Ac acetyl-tetrahydrofurospongin-1
470 R = H tetrahydrofurospongin-1

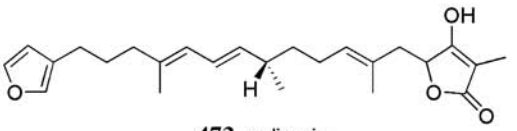
- Furanosesterterpenoids



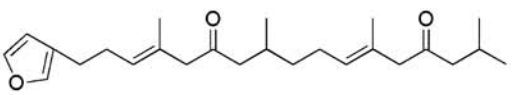
471 fasciculatin



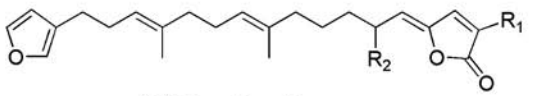
472 variabilin



473 palinurin

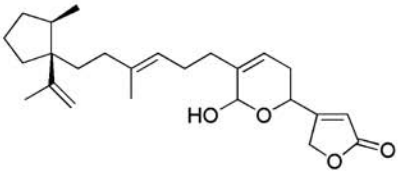


474 idiadione

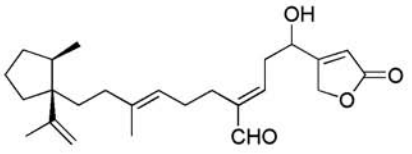


475 $R_1 = R_2 = Me$
476 $R_1 = CH_2OH$; $R_2 = Me$

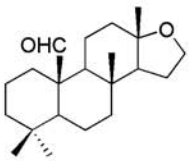
- Cyclic sesterterpenoids



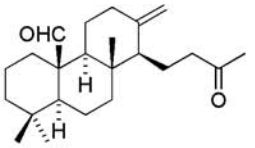
477 luffariellin-C



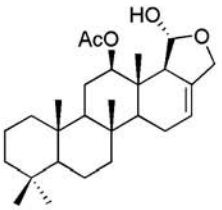
478 luffariellin-D



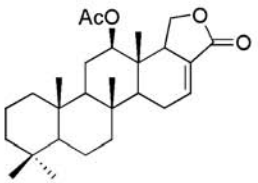
479 cadlinaldehyde



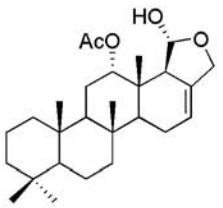
480 luteone



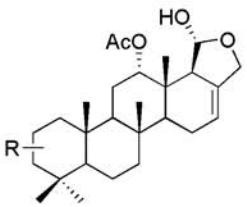
481 12-*epi*-deoxoscalarin



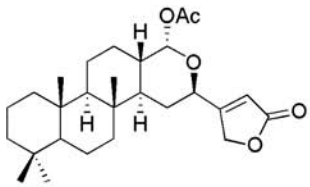
482 12-*epi*-scalarin



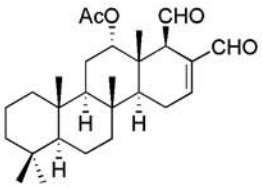
483 deoxoscalarin



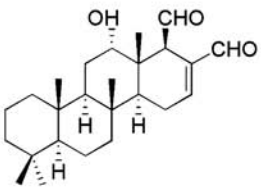
484 R = keto



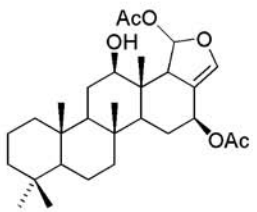
485 inorolide C



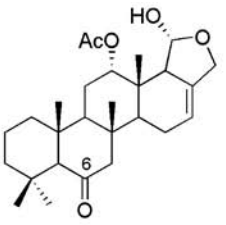
486 scalaradial



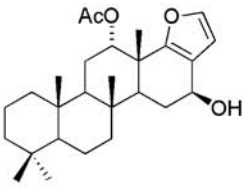
487 deacetylscalaradial



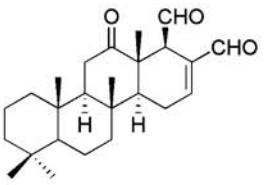
488 heteronemin



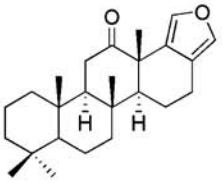
489 6-keto-deoxoscalarin



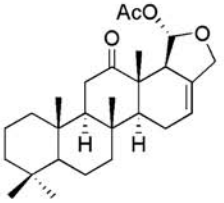
490 furoscalarol



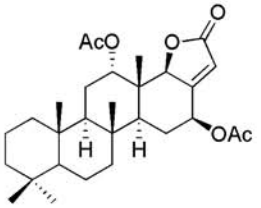
491 12-deacetoxy-12-oxo-scalaradial



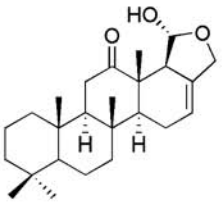
492 12,16-deacetoxy-12-oxo-scalarafuran



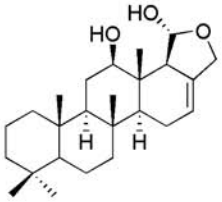
493 19-acetyl-12-deacetoxy-12--oxo-deoxoscalarin



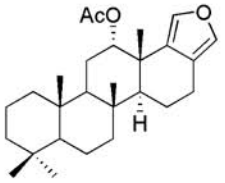
494 12α,16β-diacetoxy-scalarolbutenolide



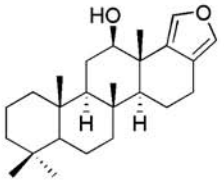
495 12-deacetoxy-12-oxo-deoxoscalarin



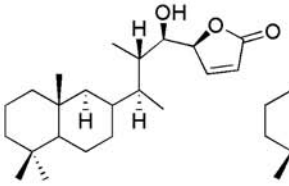
496 12-deacetyl-12-*epi*-deoxoscalarin



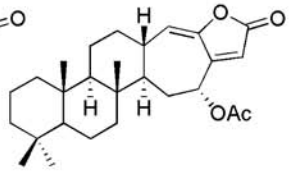
497 16-deacetoxy-12-*epi*-scalarafuran-acetate



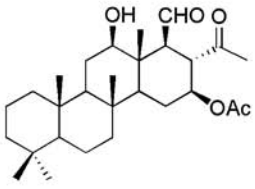
498 16-deacetoxy-scalarafuran



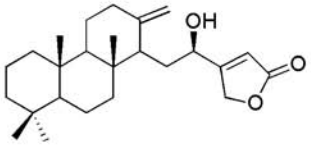
499 inorolide A



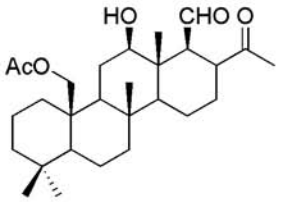
500 inorolide B



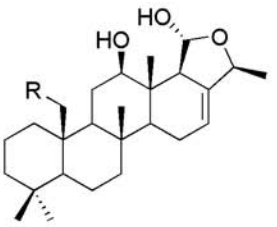
501 12,16-di-*epi*-scalarherbacin A



502 hamiltonin E

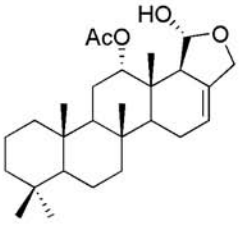


503 12-deacetyl-23-acetoxy-20-methyl-12-*epi*-scalaradial

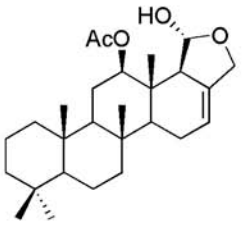


504 R = -OAc 12-deacetyl-23-acetoxy-20-methyl-12-*epi*-deoxoscalarin

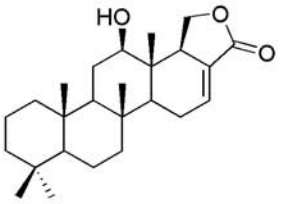
505 R = -H 12-deacetyl-20-methyl-12-*epi*-deoxoscalarin



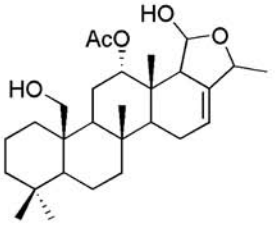
506 20-deoxoscalarin



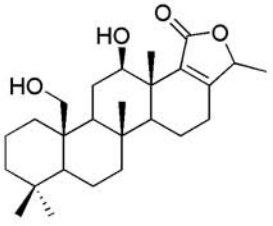
507 12-*epi*-20-deoxoscalarin



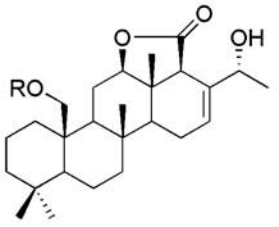
508 12-deacetyl-12-*epi*-19-dehydroxyscalarin



509 23-hydroxy-20-methyl-deoxoscalarin



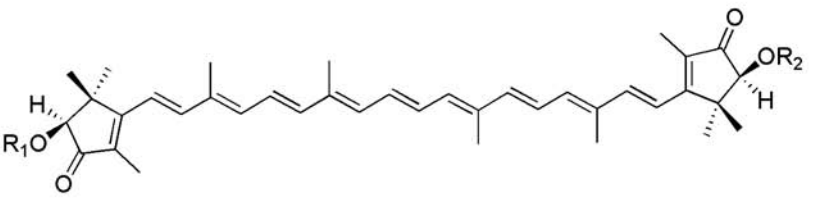
510 23-hydroxy-20-metylscalarolide



511 R = H sednolide

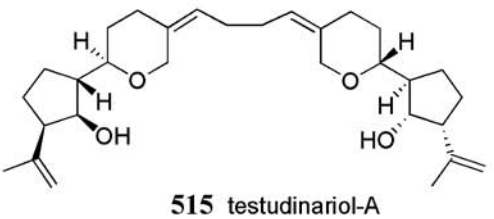
512 R = Ac sednolide 23-acetate

Triterpenoids

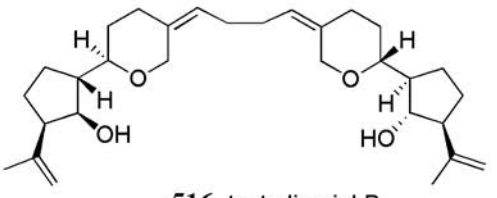


513 $R_1 + R_2$ = C27:2 + C20:1 or C20:2 + C18:0 or C20:1 + C18:1 or C19:0 + C18:1 or C18:1 + C18:0 hurghadin

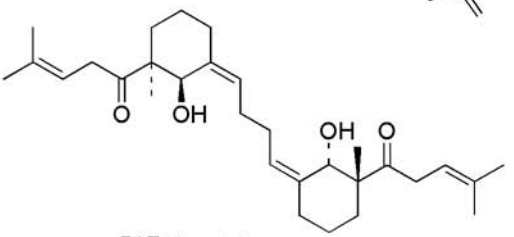
514 $R_1 + R_2 = C_{22}H_{36}$ or $C_{15}H_{32}$ or $C_{13}H_{28}$ or $C_{11}H_{24}$ actinioerythrin



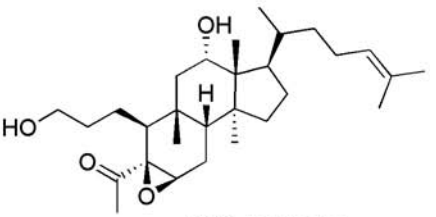
515 testudinariol-A



516 testudinariol-B



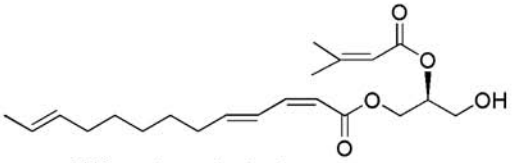
517 limatulone



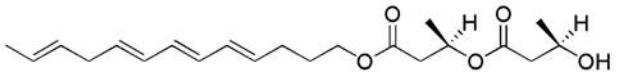
518 lovenone

Glyceride Esters

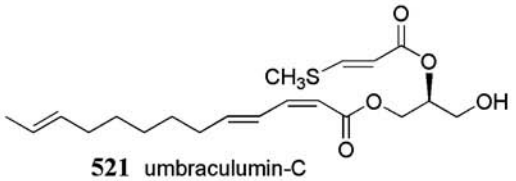
- Fatty acid esters



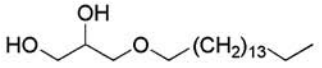
519 umbraculumin-A



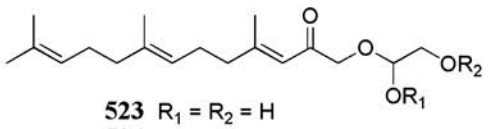
520 umbraculumin-B (no-glyceride co-occurring with **519** and **521**)



521 umbraculumin-C

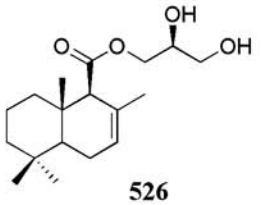


522 chimyl alcohol

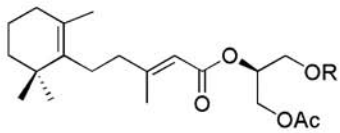


523 $R_1 = R_2 = H$
524 $R_1 = H$; $R_2 = Ac$
525 $R_1 = Ac$; $R_2 = H$

- Sesquiterpenoid esters

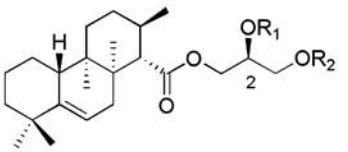


526

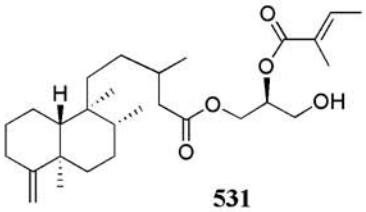


527 R = H tanyolide B
528 R = Ac tanyolide A

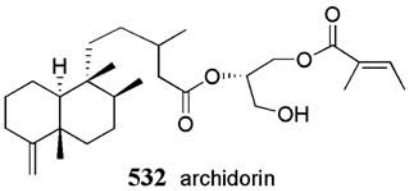
- Diterpenoid esters



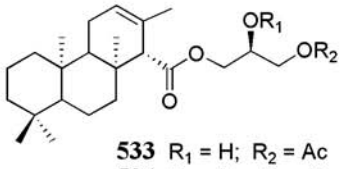
529 R_1 = H; R_2 = Ac Verrucosin A
530 R_1 = Ac; R_2 = H Verrucosin B



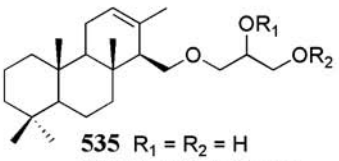
531



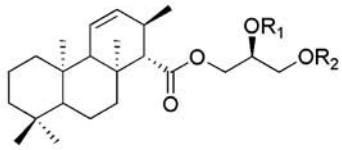
532 archidorin



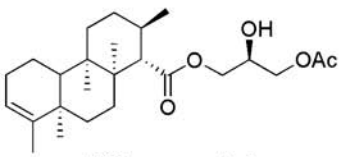
533 R_1 = H; R_2 = Ac
534 R_1 = Ac; R_2 = H



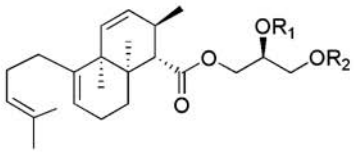
535 R_1 = R_2 = H
536 R_1 = H R 2= Ac
537 R_1 = Ac R_2 = H



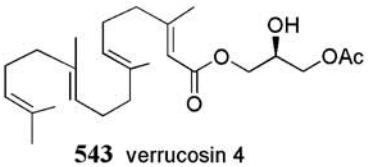
538 R_1 = H; R_2 = Ac verrucosin 1
539 R_1 = Ac; R_2 = H verrucosin 6



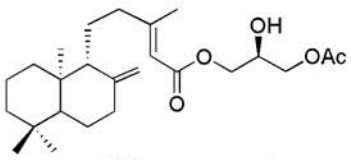
540 verrucosin 2



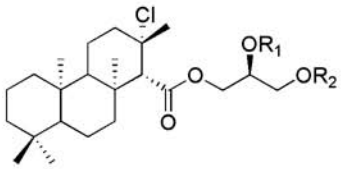
541 R_1 = H; R_2 = Ac verrucosin 3
542 R_1 = Ac; R_2 = H verrucosin 8



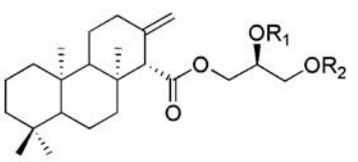
543 verrucosin 4



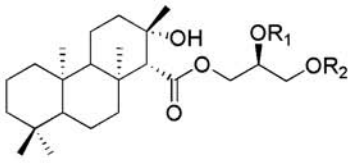
544 verrucosin 5



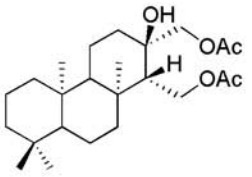
545 R_1 = H; R_2 = Ac verrucosin 7
546 R_1 = Ac; R_2 = H verrucosin 9



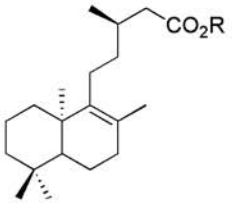
547 R_1 = H ; R_2 = Ac anisodorin-1
548 R_1 = Ac ; R_2 = H anisodorin-2



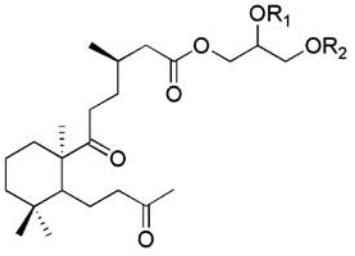
549 R_1 = H ; R_2 = Ac anisodorin-3
550 R_1 = Ac ; R_2 = H anisodorin-4



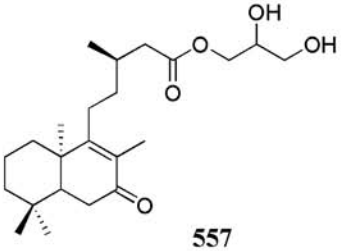
551 anisodorin-5



552 R = -CH_2-CH(OAc)-CH_2OH
553 R = -CH_2-CH(OH)-CH_2OAc
554 R =H



555 R_1 = Ac R_2 = H
556 R_1 =H R_2 = Ac



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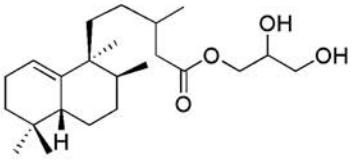
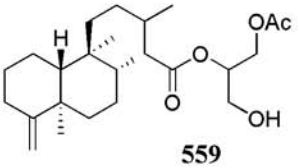


558 austrodorin



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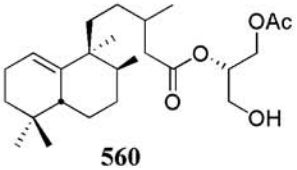
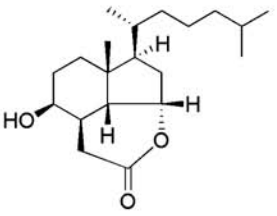
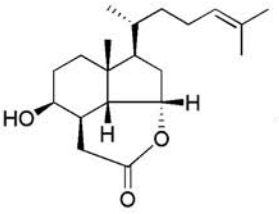


560

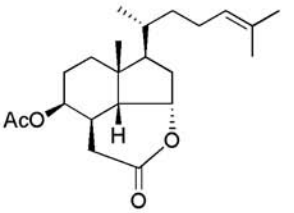
Steroids



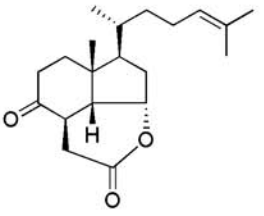
561 aplykurodin-A



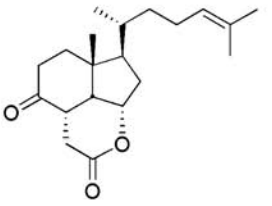
562 aplykurodin-B



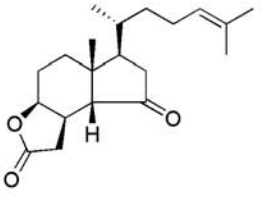
563 4-acetylaplykurodin-B



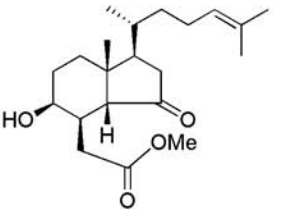
564 aplykurodinone-B



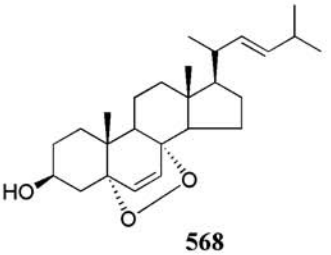
565 3-*epi*-aplykurodinone-B



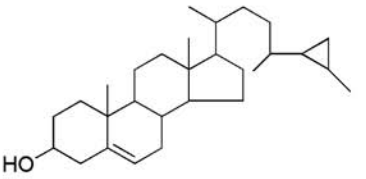
566 aplykurodinone-1



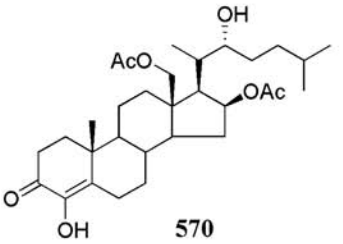
567 aplykurodinone-2



568



569 petrosterol



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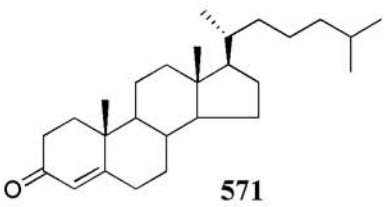

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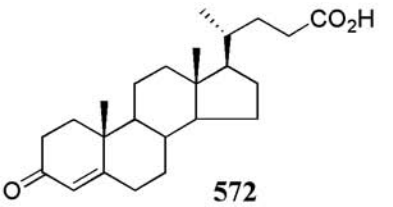

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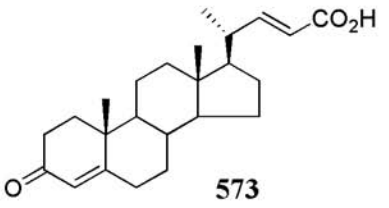

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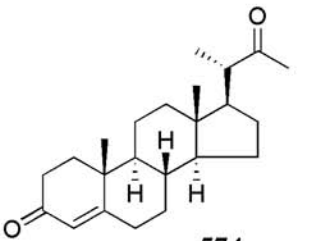

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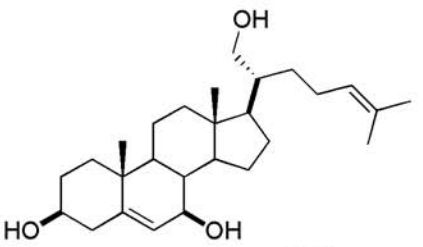

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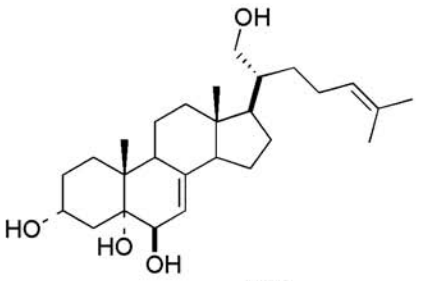

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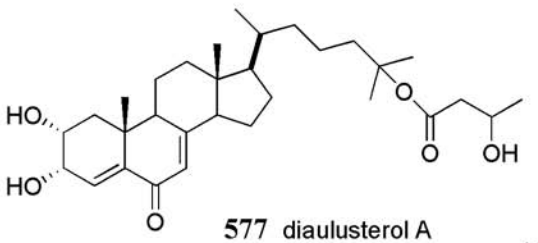
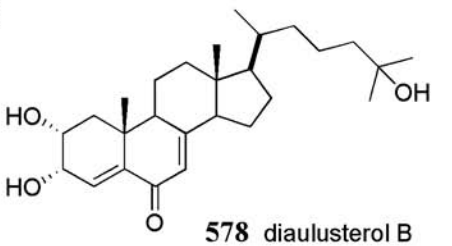
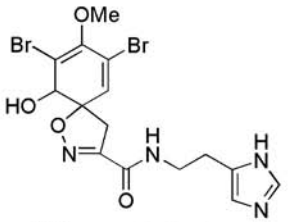


577 diaulusterol A

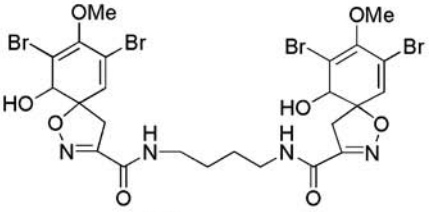


578 diaulusterol B

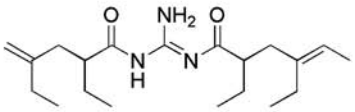
Nitrogenous coupounds



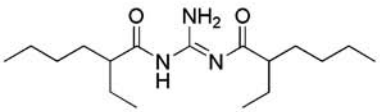
579 aerophobin-1



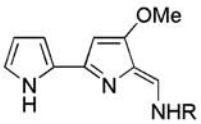
580 aerothionin



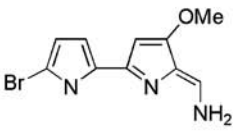
581 triophamine



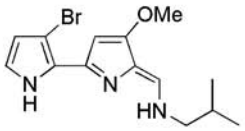
582 limaciamine



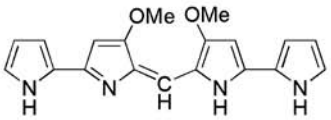
583 tambjamine-A R = H
584 tambjamine-C R =
587 tambjamine-E R =
588 tambjamine-F R =



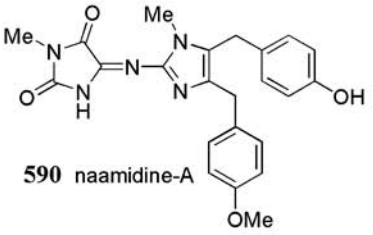
585 tambjamine-B



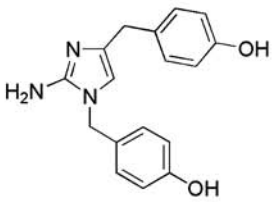
586 tambjamine-D



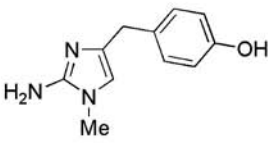
589 tetrapyrrole



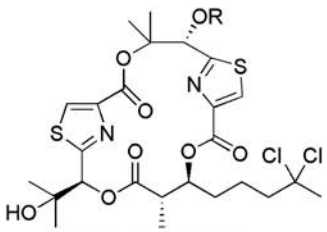
590 naamidine-A



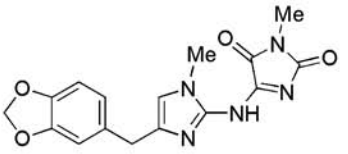
591 isonaamine-A



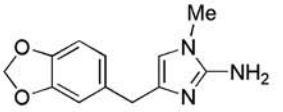
592 dorimidazole-A



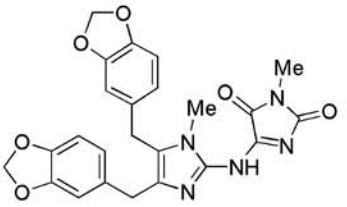
593 R = $COCH_3$
594 R = H



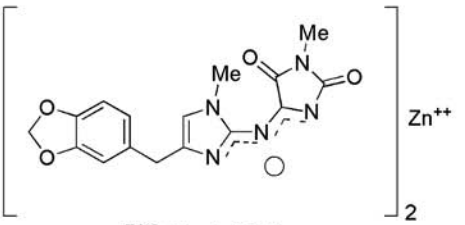
595 clathridine



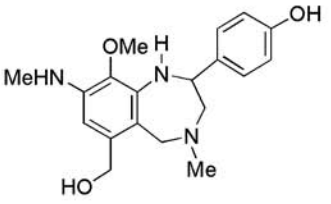
596 preclathridine-A



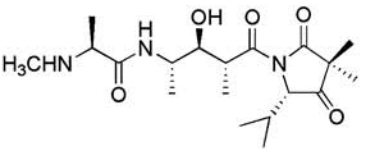
597 clathridine-B



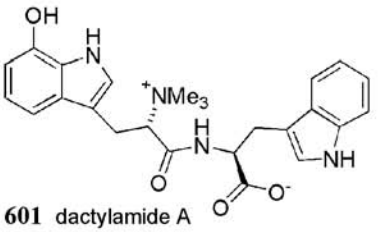
598 Zn-clathridine



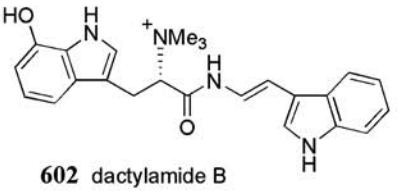
599 aplysepine



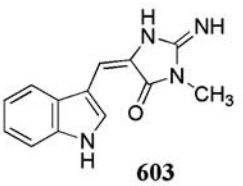
600 janolusimide



601 dactylamide A



602 dactylamide B



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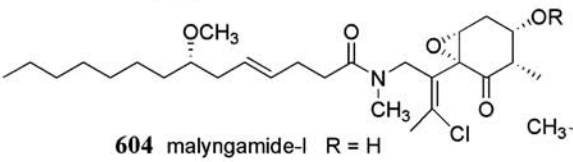
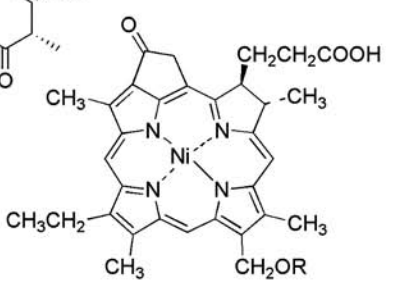
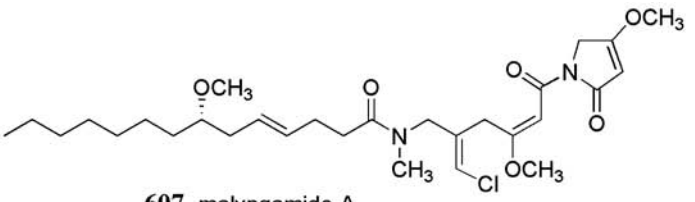


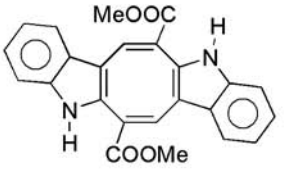
604 malyngamide-I R = H
605 acetyl malyngamide R = Ac



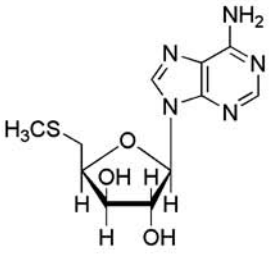
606 tunichlorin



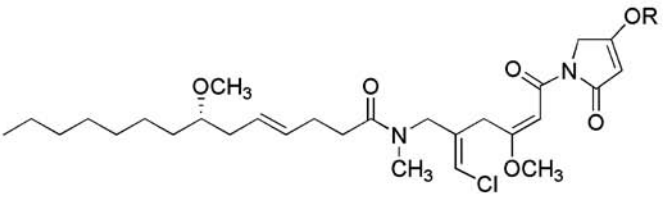
607 malyngamide-A



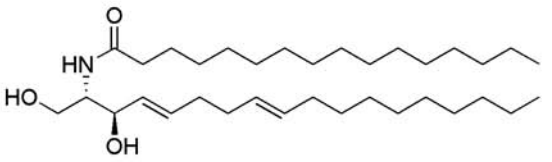
608 caulerpin



609 xylosyl MTA



610 malyngamide-B R = H
611 acetyl malyngamide-B R = Ac



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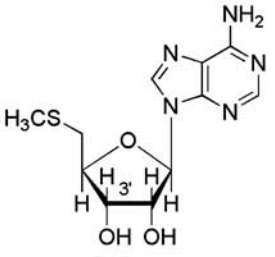
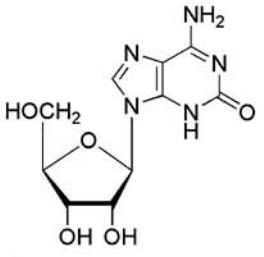
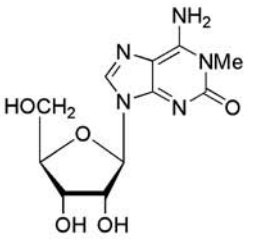


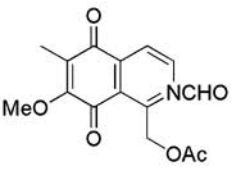
613 MTA



614 isoguanosine



615 1-methylisoguanosine



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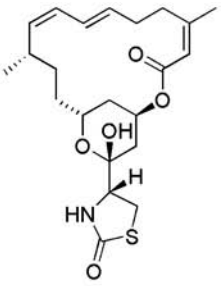
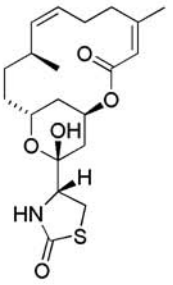
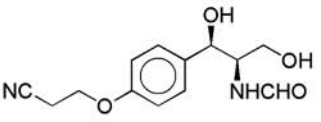


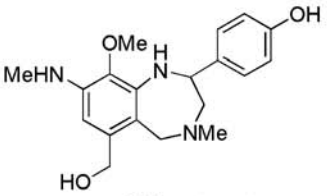
617 latrunculin A



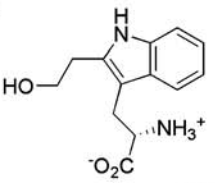
618 latrunculin B



619 bursatellin



620 aplysepine



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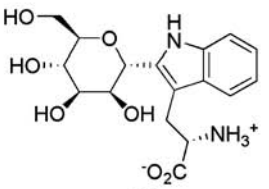

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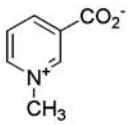

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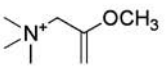

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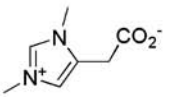

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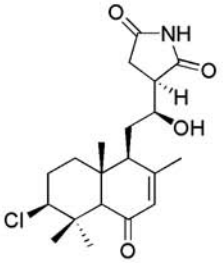

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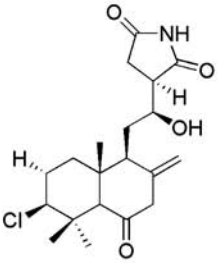

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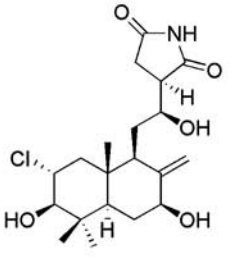

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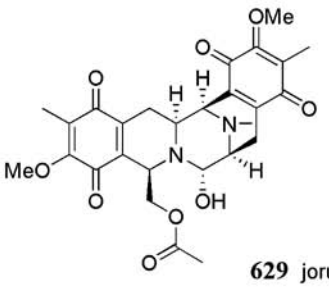
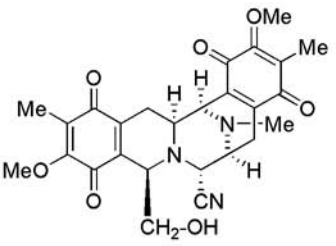
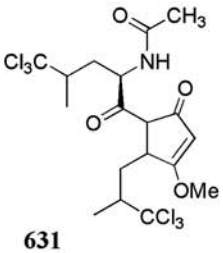


629 jorumycin



630 jorunnamycin A



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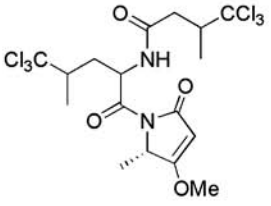
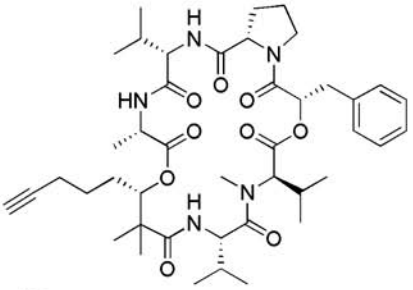
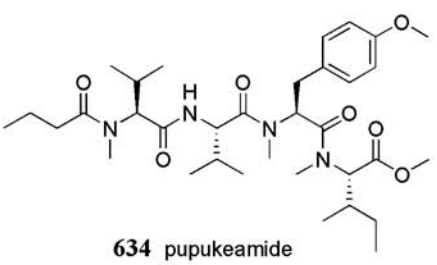


632

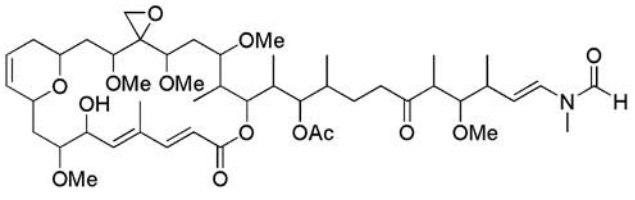
Peptides



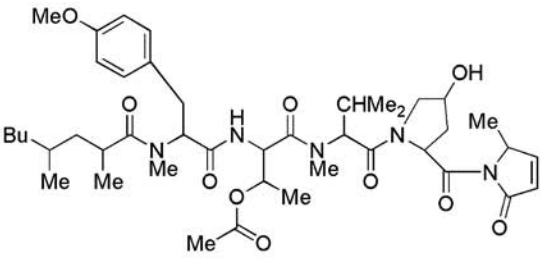
633 kulolide-1



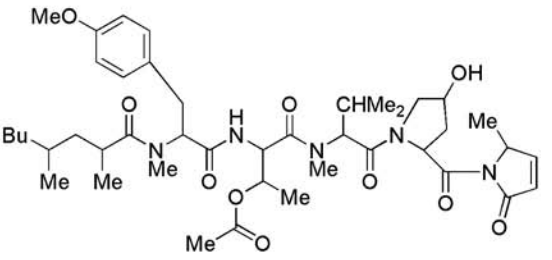
634 pupukeamide



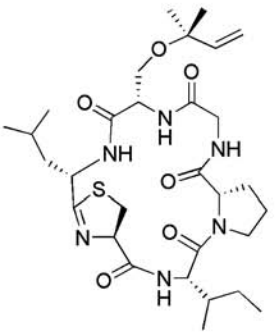
635 tolytoxin 23-acetate



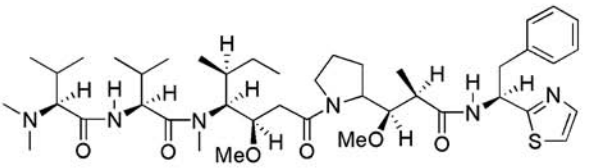
636 majusculamide D



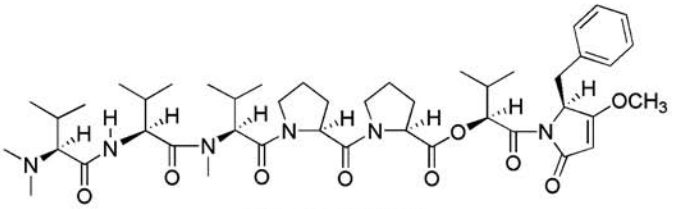
637 deoxymajusculamide D



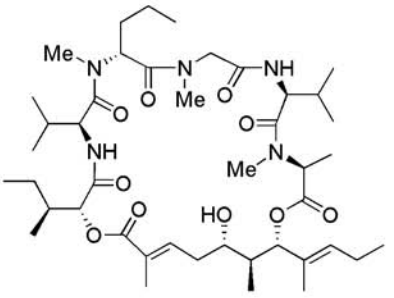
638 keenamide-A



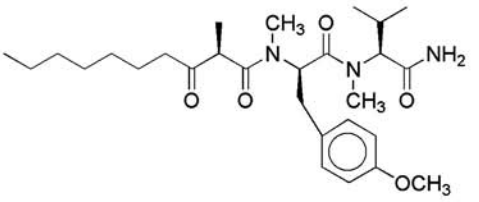
639 dolastatin 10



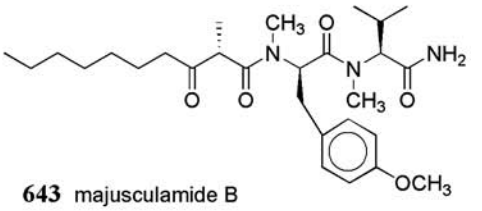
640 dolastatin 15



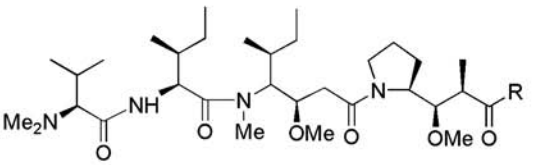
641 aurilide

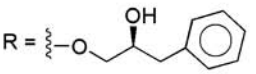


642 majusculamide A

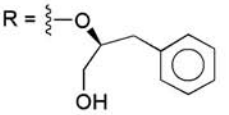


643 majusculamide B

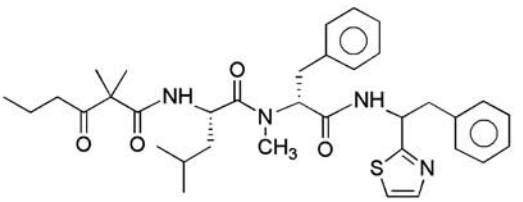




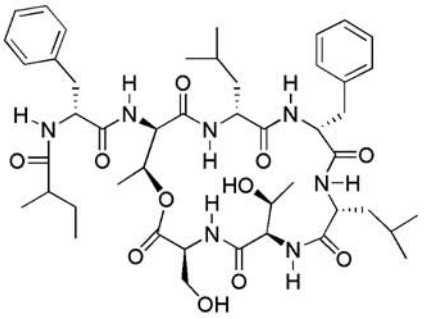
644 dolastatin-H



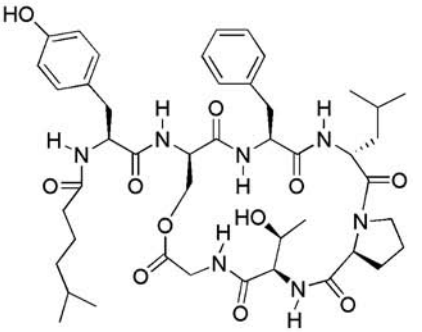
645 isodolastatin-H



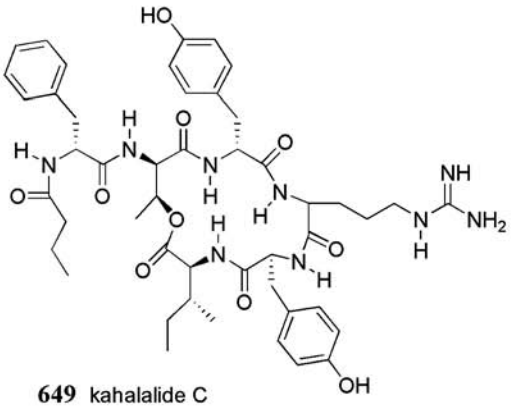
646 dolastatin 18



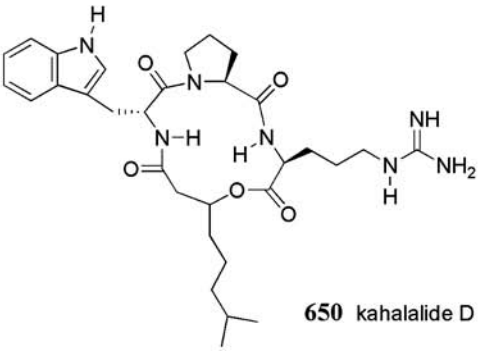
647 kahalalide A



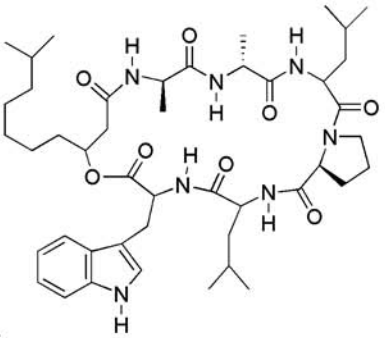
648 kahalalide B



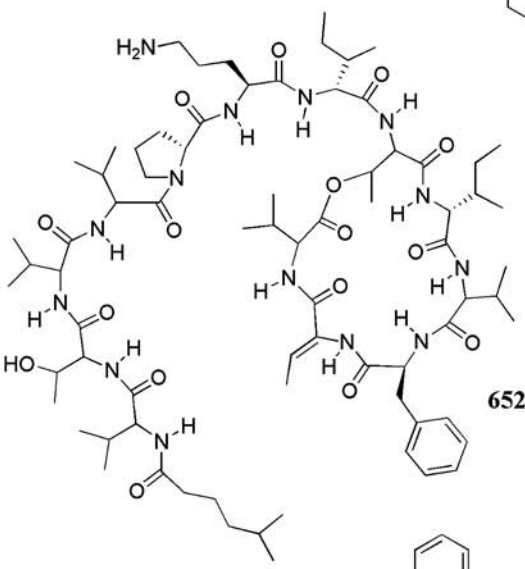
649 kahalalide C



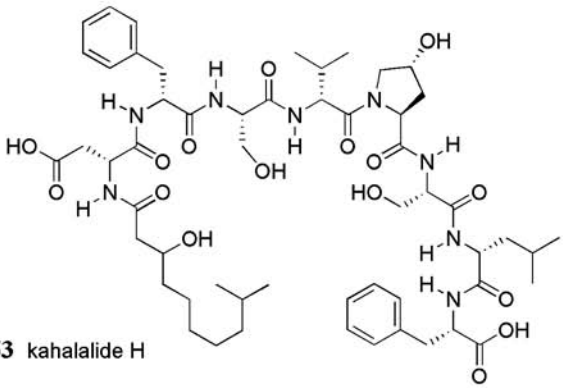
650 kahalalide D



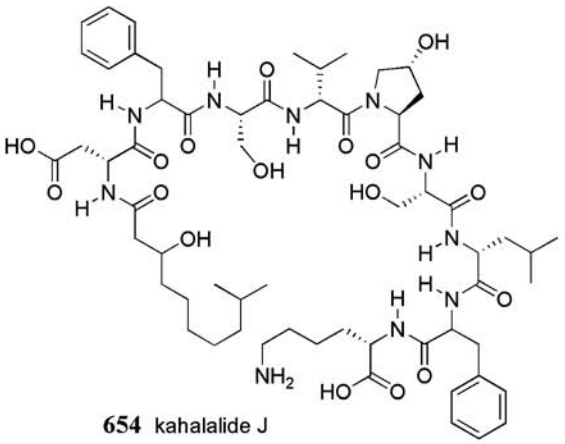
651 kahalalide E



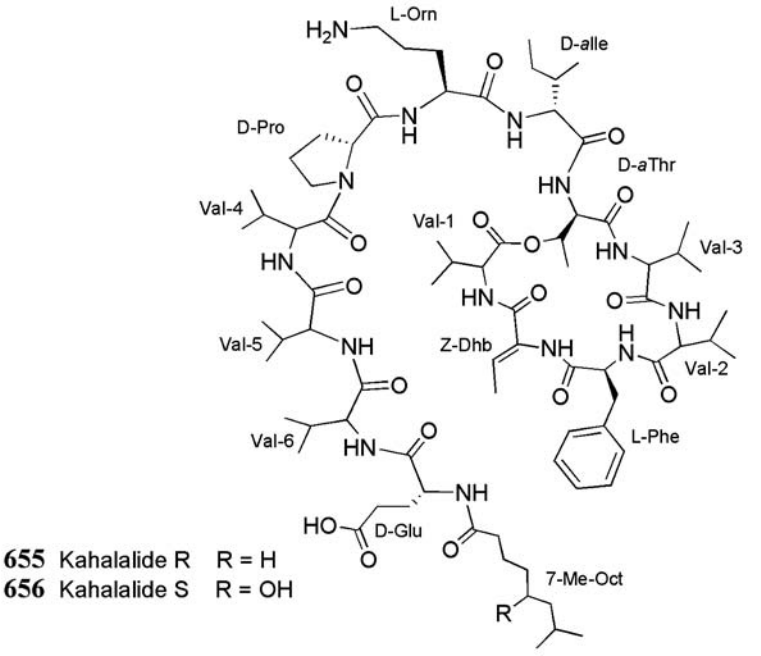
652 kahalalide F



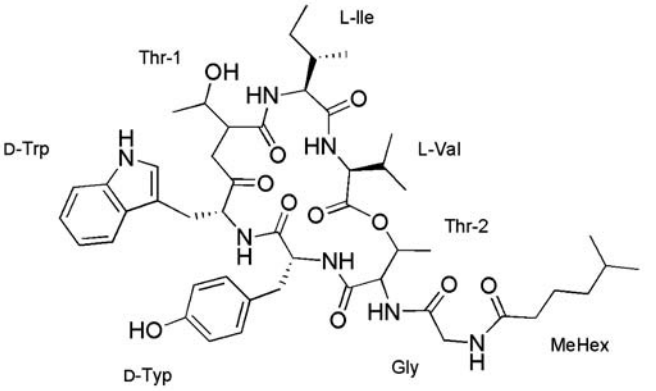
653 kahalalide H



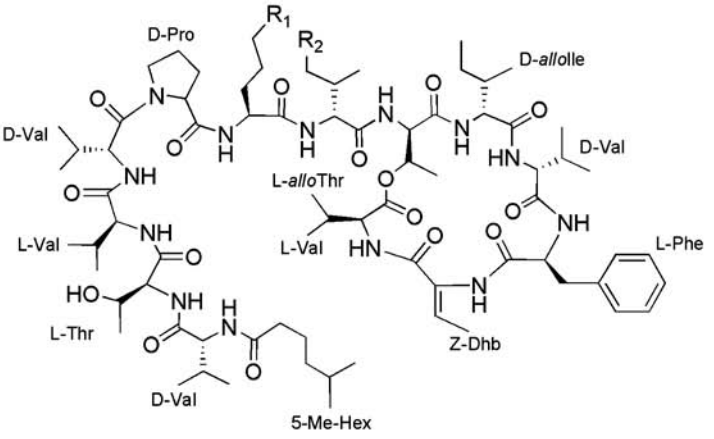
654 kahalalide J



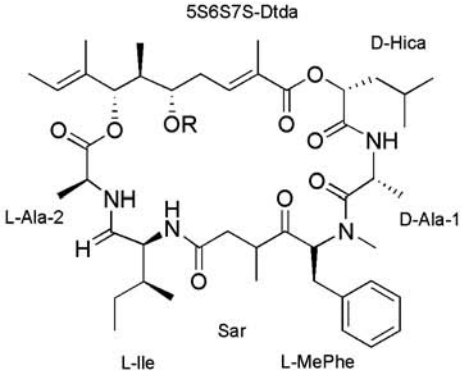
655 Kahalalide R R = H
656 Kahalalide S R = OH



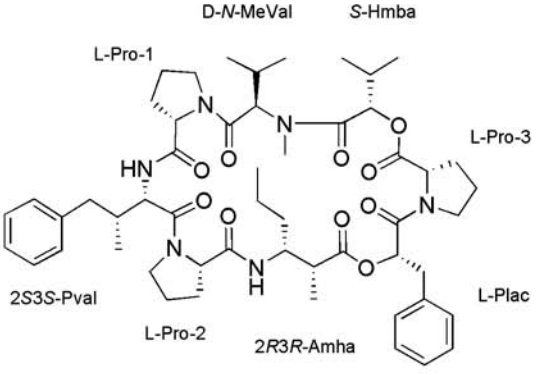
657 Kahalalide O



658 Kahalalide R R_1= NH_2; R_2 = H
659 Kahalalide S R_1 = $-CH_2NH_2$; R_2 = Me

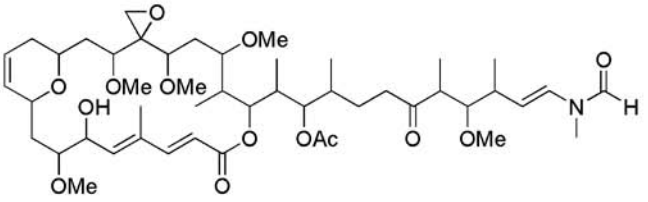


660 Kulokekahilide-2

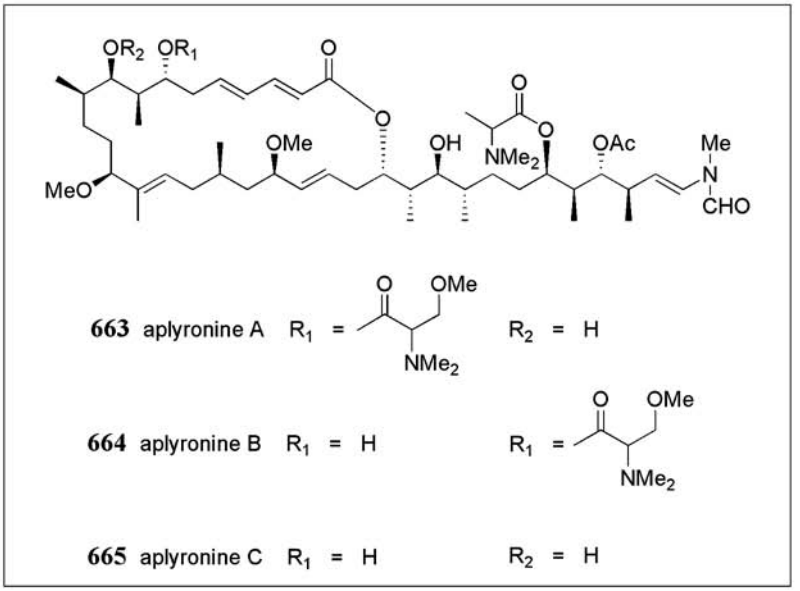


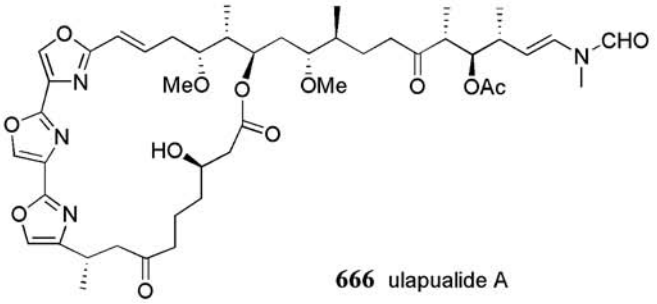
661

Macrolides

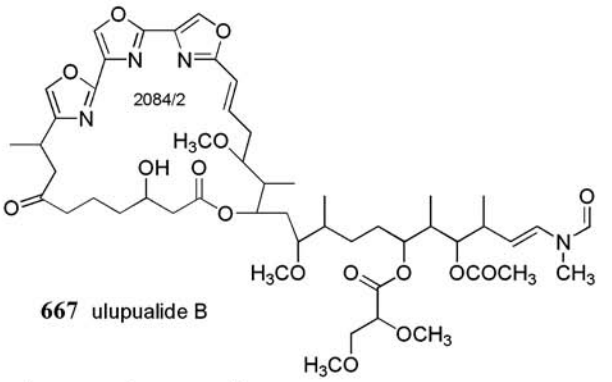


662 tolytoxin 23-acetate

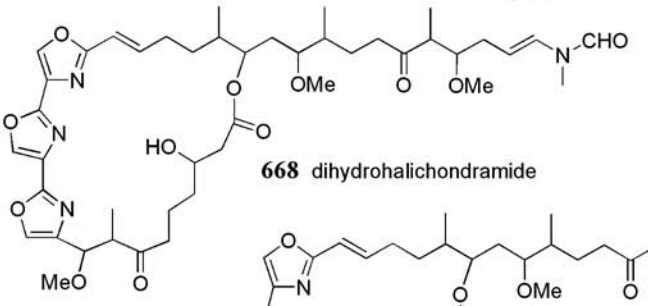




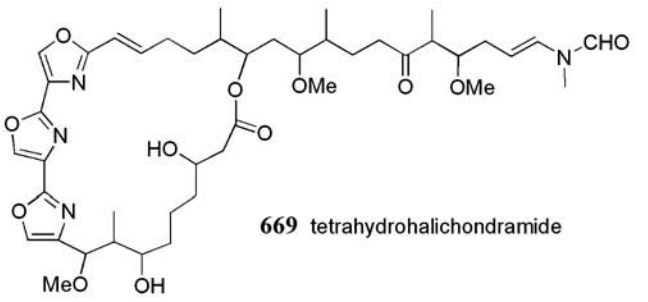
666 ulapualide A



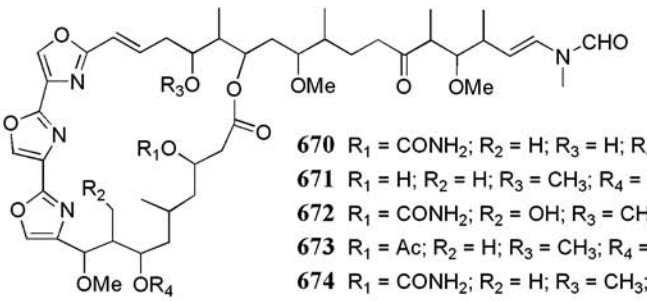
667 ulupualide B



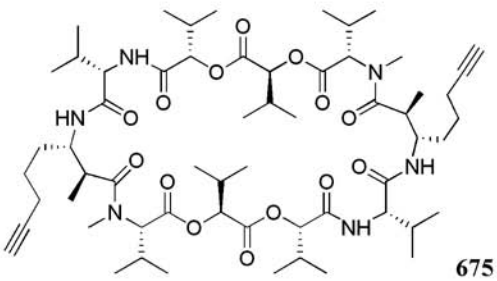
668 dihydrohalichondramide



669 tetrahydrohalichondramide



670 $R_1 = CONH_2$; $R_2 = H$; $R_3 = H$; $R_4 = H$ kabiramide A
671 $R_1 = H$; $R_2 = H$; $R_3 = CH_3$; $R_4 = H$ kabiramide B
672 $R_1 = CONH_2$; $R_2 = OH$; $R_3 = CH_3$; $R_4 = H$ kabiramide C
673 $R_1 = Ac$; $R_2 = H$; $R_3 = CH_3$; $R_4 = H$ kabiramide D
674 $R_1 = CONH_2$; $R_2 = H$; $R_3 = CH_3$; $R_4 = H$ kabiramide E



675 onchidin

Appendix II

Index to metabolite structures shown in Appendix I arranged by compound reference number, metabolite and organism in which the metabolite has been found, and reference to the literature.

Compound	Source	Reference
	Polyacetates	
	Fatty acids	
1	*Stylocheilus longicauda*	Rose et al., 1978
2	*Haminaea templadoi*	Carballeira et al., 1992
	Polyacetylenes	
3-11	*Diaulula sandiegensis*	Walker and Faulkner, 1981
12	*Discodoris atromaculata*	Castiello et al., 1980
13	*Discodoris atromaculata*	Guo et al., 1994
	Prostaglandin lactones	
14	*Tritonia* sp.	Baker and Scheuer, 1994
15-24	*Tethys fimbria*	Cimino et al., 1989, 1991 Di Marzo et al., 1991
	Cyclic acetogenins	
25	*Aplysia dactylomela*	McDonald et al., 1975
26-27	*Aplysia brasiliana*	Kinnel et al., 1979
28	*Aplysia parvula*	McPhail and Davies-Coleman, 2005
29-34	*Aplysia dactylomela*	Manzo et al., 2005
35	*Aplysia oculifera*	de Silva et al., 1983
36-37	*Aplysia oculifera*	Schulte et al., 1981
38	*Aplysia parvula*	Miyamoto et al., 1995
39	*Aplysia dactylomela*	Ciavatta et al., 1997
40	*Cadlina luteomarginata*	Burgoyne et al., 1993
41-42	*Dolabella auricularia*	Ojika et al., 1993
	Macrocyclic fatty acid lactones	
43	*Aplysia kurodai*	Ojika et al., 1990
44-48	*Aplysia depilans*	Spinella et al., 1997
49-50	*Chromodoris lochi*	Corley et al., 1988
51-54	*Dolabella auricularia*	Ojika et al., 1995; Suenaga et al., 1997
55-56	*Dolabella auricularia*	Sone et al., 1996
	Polyethers	
57-58	*Dolabella auricularia*	Suenaga et al., 1998
59-60	*Stylocheilus longicauda*	Kato and Scheuer, 1974, 1975
61	*Dolabella auricularia*	Pettit et al., 2004

Compound	Source	Reference
62	*Pinna muricata*	Chou et al., 1996
63-64	*Aplysia dactylomela*	Manzo et al., 2007
	Aromatic Polyketides	
65-67	*Navanax inermis*	Sleeper and Fenical, 1977
68	*Navanax inermis*	Sleeper et al., 1980
69	*Philinopsis speciosa*	Coval and Scheuer, 1985
70	*Bulla gouldiana*	Spinella et al., 1993
71-72	*Scaphander lignarius*	Cimino et al., 1989
73-78	*Scaphander lignarius*	Della Sala et al., 2007
79-80	*Haminoea* (*H. navicula, H. fusari, H. orteai*, *H. orbignyana)*	Cimino et al., 1991; Marin et al., 1999
81	*Haminoea callidegenita*	Spinella et al., 1998
82	*Smaragdinella calyculata*	Szabo et al., 1996
	Polypropionates	
83	*Clione antarctica*	Yoshida et al., 1995
84	*Siphonaria maura*	Manker and Faulkner, 1989
85	*Pleurobranchus membranaceus*	Ciavatta et al., 1993
86-87	*Cyerce nigricans*	Roussis et al., 1990
88-94	*Cyerce cristallina*	Di Marzo et al., 1991 Vardaro et al., 1991
95-97	*Ercolania funerea*	Vardaro et al., 1992
98-99	*Ercolania funerea*	Vardaro et al., 1992
100-103	*Placida dendritica*	Vardaro et al., 1992
104-107	*Placida dendritica*	Cutignano et al., 2003
108-111	*Aplysiopsis formosa*	Ciavatta et al., 2009
112-113	*Philinopsis speciosa*	Coval et al., 1985
114-116	*Philinopsis depicta*	Cimino et al., 1985, 1987
117	*Navanax inermis* and *Bulla gouldiana*	Spinella et al., 1993
118	*Smaragdinella calyculata*	Szabo et al., 1996
119	*Dolabrifera dolabrifera*	Ciavatta et al., 1996
120	*Dolabella auricularia*	Suenaga et al., 1996
122	*Elysia timida*	Gavagnin et al., 1994
123	*Elysia timida*	Gavagnin et al., 1994
124	*Elysia crispata*	Gavagnin et al., 1996, 1997
125-126	*Elysia diomedea*	Ireland et al., 1978
	Elysia diomedea	Ireland and Faulkner, 1981
	Placobranchus ocellatus	Ireland et al., 1979
127-128	*Elysia diomedea*	Kay et al., 1984
129-130	*Elysia chlorotica*	Dawe and Wright, 1986
131	*Placobranchus ocellatus*	Ireland et al., 1979

Compound	Source	Reference
	Elysia timida	Gavagnin et al., 1994
132-133	*Elysia crispata*	Ireland and Faulkner, 1981
134-141	*Elysia crispata*	Ksebati et al., 1985
141-143	*Placobranchus ocellatus*	Manzo et al., 2005
144-145	*Elysia diomedea*	Cueto et al., 2005
146-150	*Placobranchus ocellatus*	Fu et al., 2000
151	*Onchidium verruculatum*	Ireland et al., 1984
152-153	*Micromelo undata*	Napolitano et al., 2008
154-157	*Haminoea fusari*	Cutignano et al., 2007
158	*Peronia peronii*	Biskupak and Ireland, 1985
159	*Siphonaria serrata*	Brecknell et al., 2000
160	*Siphonaria zelandica*	Hochlowski et al., 1984
	Siphonaria denticulata	
161	*Siphonaria grisea*	Norte et al., 1988
	Phenols and Quinines	
162	*Tylodina perversa*	Cimino et al., 1984
163	*Cratena peregrina*	Ciavatta et al., 1996
164	*Dendrodoris grandiflora*	Cimino et al., 1985
165	*Doris verrucosa*	Avila et al., 1990
166-168	*Aplysia kurodai*	Kigoshi et al., 1990
169	*Mourgona germaineae*	Jensen., 1984
170	*Costasiella ocellifera*	Hay et al., 1990
171-172	*Aplysia dactylomela*	Gerwick and Whatley, 1989
173	*Aplysia dactylomela*	Kuniyoshi et al., 1985
174-176	*Asteronotus cespitosus*	Fahey and Garson, 2002
177	*Sagaminopteron nigropunctatum*	Becerro et al., 2006
	Sagaminopteron psychedelicum	
178-184	*Leminda millecra*	McPhail et al., 2001
185	*Aplysia dactylomela*	Gerwick and Whatley, 1989
	Monoterpenoids	
186-187	*Melibe leonina*	Ayer and Andersen, 1983
188-189	*Aplysia californica*	Faulkner et al., 1973
190-191	*Aplysia parvula*	Ginsbury and Paul, 2001
192-193	*Aplysia kurodai*	Miyamoto et al., 1988
194-199	*Aplysia punctata*	Ortega et al., 1997
200-201	unidentifield sea hare	Lin et al., 2001
202	*Aplysia parvula*	Grkovic et al., 2005
203	*Aplysia kurodai*	Katayama et al., 1982
	Sesquiterpenoids	
204	*Haminoea cymbalum*	Poiner et al., 1989

Compound	Source	Reference
205	*Haminoea cyanomarginata*	Mollo et al., 2008
206-208	*Oxynoe olivacea*	Cimino et al., 1990; Gavagnin et al., 1994
	Lobiger serradifalci	
	Ascobulla sp.	
	Lobiger souverbiei	Gavagnin et al., 2000
	Oxynoe antillarium	
209-210	*Ascobulla ulla*	Gavagnin et al., 2000
211-212	*Volvatella* sp.	Fontana et al., 1999
213-214	*Elysia expansa*	Ciavatta et al., 2006
215-216	*Elysia crispata*	Gavagnin et al., 1997
217	*Onchidella binneyi*	Ireland and Faulkner, 1978
218-219	*Tochuina tetraquetra*	Williams and Andersen, 1987
220-223	*Leminda millecra*	Pika and Faulkner, 1994
224	*Aplysia dactylomela*	Schmitz and McDonald, 1974
225	*Aplysia brasiliana*	Stallard et al., 1978
226	*Dolabella ecaudata*	Pettit et al., 1980
227-228	*Acanthodoris nanaimoensis*	Ayer et al., 1984
229	*Acanthodoris brunnea*	Faulkner et al., 1990
230-231	*Asteronotus cespitosus*	Fahey and Garson, 2002
232-234	*Dermatobranchus otome*	Ishibashi et. al., 2006
235	*Dendrodoris tuberculosa*	Okuda et al., 1983
	Dendrodoris krebsii	
	Dendrodoris nigra	
236-237	*Dendrodoris limbata*	Cimino et al., 1982, 1985
	Dendrodoris grandiflora	
238	*Dendrodoris grandiflora*	Cimino et al., 1985
239	*Dendrodoris limbata*	Avila et al., 1991
	Dendrodoris grandiflora	
240	*Dendrodoris denisoni*	Grkovic et al., 2005
241	*Dendrodoris arborescens*	Fontana et al., 1999
242	*Dendrodoris albopunctata*	Okuda et al., 1983
243	*Bathydoris hodgsoni*	Iken et al., 1998
244	*Cadlina luteomarginata*	Dumdei et al., 1997
245-246	*Cadlina luteomarginata*	Hellou et al., 1981
		Gustafson et al., 1985
247	*Chromodoris* molluscs	Schulte et al., 1982
248-249	*Austrodoris kerguelenensis*	Gavagnin et al., 2003
250-251	*Leminda millecra*	McPhail et al., 2001
252-265	*Dendrodoris carbunculosa*	Sakio et al., 2001
	Halogenated sesquiterpenoids	
266	*Aplysia angasi*	Pettit et al., 1977
267-272	*Aplysia dactylomela*	McPhail et al., 1999
273	*Aplysia kurodai*	Yamamura and Hirata, 1963

Compound	Source	Reference
274	*Aplysia dactylomela*	Ichiba and Higa, 1986
275-276	*Aplysia dactylomela*	Baker et al., 1988
277-278	*Aplysia dactylomela*	Kaiser et al., 1998
279	*Aplysia dactylomela*	Schmitz et al., 1980
280-281	*Aplysia brasiliana*	Dieter et al., 1979
282	*Aplysia parvula*	Grkovic et. al., 2005
	Isocyanosesquiterpenoids	
283-284	*Phyllidia varicosa*	Burreson et al., 1975 Hagadone et al., 1979
285-289	*Phyllidia pustulosa*	Fusetani et al., 1991
290-294	*Phyllidia varicosa* *Phyllidia pustulosa*	Kassühlke et al., 1991
295-298	*Phyllidia ocellata*	Fusetani et al., 1992
299-302 *P*	*hyllidia ocellata* *Phyllidia varicosa* *Phyllidia pustulosa* *Phillidiopsis krempfi*	Okino et al., 1996
303-305	*Phyllidiella pustulosa*	Manzo et al., 2004
306	*Phyllidia pustulosa*	Hirota et al., 1998
307-312	*Cadlina luteomarginata*	Burgoyne et al., 1993
313-314	*Phyllidia varicosa*	Yasman et al., 2003
315-316	*Hexabranchus sanguineus*	Zhang et al., 2007
	Furanosesquiterpenoids	
317-318	*Doriopsilla pelseneeri*	Gaspar et al., 2005
319	*Tritonia hamnerorum*	Cronin et al., 1995
320-325	*Chromodoris epicuria* *Ceratosoma brevicaudatum*	Ksebati and Schmitz, 1988
326	*Tyrinna nobilis*	Fontana et al., 1998
327-330	*Hypselodoris villafranca* *Hypselodoris cantabrica* *Hypselodoris tricolor*	Fontana et al., 1993
331-332	*Hypselodoris tricolor*	Fontana et al., 1994
333-337	*Hypselodoris picta*	Fontana et al., 1994
338	*Hypselodoris capensis*	McPhail et al., 1998
339-342	*Doriopsilla areolata*	Spinella et al., 1994
343-344	*Cadlina luteomarginata*	Thompson et al., 1982
345-346	*Cadlina laevis*	Fontana et al., 1995
347-349	*Cadlina pellucida*	Fontana et al., 1995
350	*Dendrodoris krebsii* *Doriopsilla albopunctata* *Doriopsilla areolata*	Gavagnin et al., 2001

Compound	Source	Reference
	Diterpenoids	
351-352	*Pleurobranchaea meckelii*	Ciavatta et al., 1995
353	*Trimusculus reticulatus*	Manker and Faulkner, 1987
354-357	*Tochuina tetraquetra*	Williams and Andersen, 1987
358	*Tochuina tetraquetra*	Wratten et al., 1977
359-361	*Armina maculata*	Guerriero et al. 1987
362	*Phyllodesmium guamensis*	Slattery et al., 1998
363	*Phyllodesmium longicirra*	Coll et al., 1985
364	*Aplysia kurodai*	Yamamura and Hirata, 1971
365	*Aplysia kurodai*	Ojika et al., 1990
366-367	*Aplysia dactyomela*	Schmitz et al., 1979
368	*Aplysia dactyomela*	Gonzalez et al., 1987
369	*Dolabella* and *Aplysia*	Estrada et al., 1989
370	*Aplysia depilans*	Minale and Riccio, 1976
371	*Aplysia vaccaria*	Midland et al., 1983
372	*Aplysia juliana*	Atta-ur Rahman et al., 1991
373	*Dolabella californica*	Ireland et al., 1976 Ireland and Faulkner, 1977
374	*Dolabella auricularia*	Pettit et al., 1976
375	*Elysia translucens*	Gavagnin et al., 1994
376-377	*Bosellia mimetica*	Gavagnin et al., 1994
378-380	*Thuridilla hopei*	Gavagnin et al., 1993
381	*Pseudochlorodesmis furcellata*	Paul et al., 1988
382	*Elysia halimedae*	Paul and Van Alstyne, 1988
383	*Cyerce nigricans*	Roussis et al., 1990
384	*Chromodoris norrisi*	Hochlowski et al., 1983
385	*Chromodoris sedna*	Hochlowski et al., 1983
386-389	*Cadlina luteomarginata*	Tischler et al., 1989 Tischler and Andersen, 1991
390-393	*Cadlina luteomarginata*	Dumdei et al., 1997
394-395	*Chromodoris cavae*	Dumdei et al., 1989 Morris et al., 1991
396-405	*Chromodoris gleniei* *Chromodoris geminus* *Chromodoris annulata* *Chromodoris inopinata*	de Silva et al., 1991
406	*Chromodoris ghiselini*	Hochlowski et al., 1982
407-416	*Chromodoris obsoleta*	Miyamoto et al., 1996
417	*Chromodoris macfarlandi*	Molinski et al., 1986
418-422	*Chromodoris luteorosa*	Cimino et al., 1990 Gavagnin et al., 1992
423-428	*Chromodoris hamiltoni*	Pika and Faulkner, 1995 McPhail et al., 1997
429-437	*Glossodoris atromarginata*	Fontana et al., 1997

Compound	Source	Reference
438-439	*Glossodoris atromarginata*	Somerville et al., 2006
440-448	*Chromodoris epicuria*	Ksebati et al., 1988
449	*Tyrinna nobilis*	Fontana et al., 1998
450-453	*Syphonota geographica*	Carbone et al., 2008
454-457	*Phyllidia pustulosa*	Manzo et al., 2004
458	unidentified nudibranch	Van Wyk et al., 2007
459	*Hexabranchus sanguineus*	Zhang et al., 2007
460-463	*Trimusculus peruvianus*	Diaz-Marrero et al., 2003
464	*Trimusculus reticulatus*	Manker and Faulkner, 1987
	Sesterterpenoids	
	Degraded furanosesterterpenoids	
464-466	*Cadlina pellucida*	Fontana et al., 1995
467-470	*Dendrodoris grandiflora*	Cimino et al., 1985
	Furanosesterterpenoids	
471-473	*Discodoris indecora*	Marin et al., 1997
474	*Cadlina luteomarginata*	Thompson et al., 1982
475-476	*Hypselodoris capensis*	McPhail et al., 1998
	Cyclic sesterterpenoids	
477-478	*Chromodoris funerea*	Kernan et al., 1988
479	*Cadlina luteomarginata*	Dumdei et al., 1997
480	*Cadlina* molluscs	Hellou et al., 1981, 1982
481	*Cadlina pellucida*	Fontana et al., 1995
482-484	*Chromodoris funerea*	Kernan et al., 1988
485	*Chromodoris inornata*	Miyamoto et al., 1992
486	*Glossodoris pallida*	Avila and Paul, 1977
487-488	*Glossodori cincta* *Glossodoris hikeurensis* *Glossodoris pallida*	Rogers and Paul, 1991
489-490	*Hypselodoris orsini* *Glossodoris rufomarginata*	Cimino et al., 1993 Gavagnin et al., 2004
491-498	*Glossodoris* spp.	Manzo et al., 2007
499-500	*Chromodoris inornata*	Miyamoto et al., 1992
501	*Glossodoris sedna*	Hochlowski et al., 1983
502	*Chromodoris hamiltoni*	Pika and Faulkner, 1995 McPhail et al., 1997
503-508	*Glossodoris dalli* *Glossodoris sedna*	Fontana et al., 2000
509-512	*Glossodoris sedna*	Hochlowski et al., 1983
	Triterpenoids	
513-514	*Hexabranchus sanguineus*	Guo et al., 1998

Compound	Source	Reference
515-516	*Pleurobranchus testudinarius*	Spinella et al., 1997
517	*Lottia limatula*	Albizati et al., 1985
518	*Adalaria loveni*	Graziani et al., 1995
	Glyceride Esters	
	Fatty acid esters	
519-521	*Umbraculum umbraculum*	Cimino et al., 1988
522	*Tritoniella belli*	McClintock et al., 1994
	Sesquiterpenoid esters	
523-526	*Doris odhneri*	
	Doris montereyensis	Andersen et al., 1980
		Gustafson et al., 1985
527-528	*Doris tanya*	Krug et al., 1995
	Diterpenoid esters	
529-530	*Doris verrucosa*	Cimino et al., 1988
		Gavagnin et al., 1997
531	*Archidoris pseudoargus*	Soriente et al., 1993
532	*Archidoris tuberculata*	Cimino et al., 1993
533-534	*Anisodoris fontaini*	Zubia et al., 1993
	Doris verrucosa	Gavagnin et al., 1997
535-537	*Doris odhneri*	Andersen et al., 1980
	Doris montereyensis	Gustafson et al., 1985
538-546	*Doris verrucosa*	Gavagnin et al., 1997
547-551	*Anisodoris fontaini*	Gavagnin et al., 1999
552-557	*Austrodoris kerguelensis*	Davies-Coleman et al., 1991
558	*Austrodoris kerguelensis*	Gavagnin et al., 1995
559-560	*Austrodoris kerguelensis*	Gavagnin et al., 2003
	Steroids	
561-562	*Aplysia kurodai*	Miyamoto et al., 1986
563-564	*Aplysia fasciata*	Spinella et al., 1992
565	*Aplysia fasciata*	Ortega et al., 1997
566-567	*Syphonota geographica*	Gavagnin et al., 2005
568	*Adalaria* sp.	Stonard et al., 1980;
569	*Peltodoris atromaculata*	Castiello et al.,1978
570	*Hervia peregrina,*	Cimino et al., 1980
	Flabellina affinis,	
	Coryphella lineata,	
	Eudendrium ramosum	
571-573	*Aldisa sanguinea*	Ayer et al., 1982
574	*Aldisa smaragdina*	Gavagnin et al., 2002
575-576	*Trimusculus peruvianus*	Diaz-Marrero et al., 2003

Compound	Source	Reference
577-578	*Diaulula sandiegensis*	Williams et al., 1986
	Nitrogenous compounds	
579-580	*Tylodina perversa*	Teeyapant et al., 1993
581	*Triopha catalinae* and *Polycera tricolor*	Gustafson and Andersen, 1982, 1985
582	*Limacia clavigera*	Graziani and Andersen, 1998
583-586	*Tambjia abdere* and *Tambjia. eliora*	Carté and Faulkner, 1983, 1986
587-588	*Nembrotha cristata* *Nembrotha kubaryana*	Lindquist and Fenical, 1991
589	*Nembrotha* spp.	Paul et al., 1990
590-592	*Aegires (=Notodoris) gardineri*	Alvi et al., 1991
593-594	*Bursatella leachii*	Suntornchashwej et al., 2005
595	*Aegires (=Notodoris) gardineri*	Carroll et al., 1993
596-598	*Aegires (=Notodoris) gardineri*	Alvi et al., 1993
599	*Aplysia kurodai*	Ojika et al., 1993
600	*Janolus cristatus*	Sodano and Spinella, 1986
601-602	*Aplysia dactylomela*	Grkovic et al., 2005
603	*Phestilla melanobrachia*	Okuda et al., 1982
604-605	*Stylocheilus longicauda*	Todd and Gerwick, 1995 Rose et al., 1978
606	*Dolabella auricularia*	Pettit et al., 1993
607	*Stylocheilus longicauda*	Paul and Pennings, 1991
608	*Lobiger souverbie* *Oxynoe antillarium*	Gavagnin et al., 2000
609	*Doris verrucosa*	Cimino et al., 1986
610-611	*Stylocheilus longicauda*	Paul and Pennings, 1991
612	*Armina babai*	Ishibashi et al., 2006
613	*Doris verrucosa*	Cimino et al., 1986
614	*Diaulula sandiegensis*	Fuhrman et al., 1981
615	*Peltodoris nobilis*	Kim et al., 1981
616	*Jorunna funebris*	de Silva et al., 1988
617	*Chromodoris elisabethina*	Okuda and Scheuer, 1985
618	*Glossodoris quadricolor*	Mebs et al., 1985
619	*Bursatella leachii leachii* *Bursatella leachii savignyana*	Cimino et al., 1987
620	*Aplysia kurodai*	Ojika et al., 1993
621-625	*Halgerda aurantiomaculata*	Fahey et al., 2007
626-628	*Pleurobranchus forskalii* *Pleurobranchus albiguttatus*	Fu et al., 2004
629	*Jorunna funebris*	Fontana et al., 2000
630	*Jorunna funebris*	Charupant et al., 2007
631-632	*Asteronotus cespitosus*	Fahey and Garson, 2002

Compound	Source	Reference
	Peptides	
633	*Philinopsis speciosa*	Reese et al., 1996
634	*Philinopsis speciosa*	Nakao et al., 1996
635	*Philinopsis speciosa*	Nakao et al., 1998
636-637	*Lyngbya majuscula*	Moore and Entzeroth, 1988
638	*Pleurobranchus forskalii*	Wesson and Hamann., 1996
639-640	*Dolabella auricularia*	Pettit et al., 1993
641	*Dolabella auricularia*	Suenaga et al., 1996
642-643	*Stylocheilus longicauda*	Pennings et al., 1996
644-645	*Dolabella auricularia*	Sone et al., 1996
646	*Dolabella auricularia*	Pettit et al., 1997
647-654	*Elysia rufescens*	Hamann et al., 1993 Goetz et al., 1997
655-656	*Elysia grandifolia*	Ashour et al., 2006
657	*Elysia ornata*	Horgen et al., 2000
658-659	*Elysia grandifolia*	Tilvi and Naik, 2007
660	*Philinopsis speciosa*	Nakao et al., 2004
661	*Philinopsis speciosa*	Kimura et al., 2002
	Macrolides	
662	*Philinopsis speciosa*	Nakao et al., 1998
663-665	*Aplysia kurodai*	Yamada et al., 1993
666-667	*Hexabranchus sanguineus*	Roesener et al., 1986
668-674	*Hexabranchus sanguineus*	Kernan and Faulkner, 1988 Matsunaga et al., 1989
675	*Onchidium* sp.	Rodriguez et al., 1994