Some Marine Ecological Phenomena: Chemical Basis and Biomedical Potential

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Analysis of secondary metabolites derived from marine organisms has revealed a broad spectrum of novel molecular architecture. The function of these compounds in their natural habitat is linked to various aspects of species survival, and the compounds have also served as characteristic chemical markers through successive trophic levels. Fundamental questions concerning the locus of synthesis in complex and intricate assemblies of plants and animals and the pathways of biosynthesis are beginning to be answered. It is now apparent that the marine environment gives rise to some distinctive chemistry, which is generated along characteristic pathways. Some of the newly described compounds have already become valuable tools in biomedicine.

THE PHYSICAL AND CHEMICAL PROPERTIES OF THE MARINE environment, which profoundly influence the biology in the ocean, are quite different from those of the terrestrial environment. Notable among these differences are (i) the greater density of the medium (seawater versus air), which makes possible the existence and chemical communication of large floating communities of plants and animals, predominantly microscopic in size; (ii) reduced absorption of light, which permits photosynthesis in only a narrow surface zone; and (iii) the prevalence among marine biota of protein-dominated rather than carbohydrate-dominated skeletal materials. Because of these properties, there is a greater complexity in the food chain in the marine environment than in the terrestrial environment. These properties have also resulted in an abundance of filter-feeding sessile organisms, which in turn serve as excellent substrates for epibionts and symbionts, communities that are either absent or rare in terrestrial ecosystems.

Organic chemists, who during the last 30 years have intensively studied the chemistry of marine organisms, have begun to probe the molecular basis and the biomedical potential of some of the relations that are characteristic of the marine environment. Early research targets for the study of secondary marine metabolites were the well-known seafood toxins tetrodotoxin and saxitoxin. With researchers developing an increasing interest in marine research, many biota were sampled in anticipation of new chemistry or desirable physiological activity. Among notable results of that period were the discovery of sponge nucleosides containing the rare sugar arabinose (*1*), which eventually led to development of the antiviral drug vidabarine (ara-A); marketing of the insecticide padan, an analog of nereistoxin, a metabolite of an annelid (*2*); isolation of prostaglandins from a gorgonian coral, where they occur in high concentration (*3*); and the elucidation of marine steroids that have uncommon structural elements (*4*). As more chemists collaborated with marine biologists and started to dive, they had the opportunity to observe ecological phenomena. As a result, more sophisticated research targets came under study with the realization that many observed intra- and interspecific phenomena might have a molecular basis. In this article, I discuss (i) some examples of these phenomena to illustrate the role of chemistry in species survival; (ii) some of the biomedically significant by-products of this research; (iii) avenues toward discovering the origin of secondary marine metabolites, whether synthesized by an epibiont, by the host, or jointly; and (iv) experimental approaches to elucidate the biosynthetic pathways in some sessile marine invertebrates. The discussion is not comprehensive. Instead, it draws primarily on a few examples that I am most familiar with to highlight general points.

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Defense and Predation

Sea urchins, which are echinoderms that have calcareous shells, and many mollusks that have shells into which they can retreat are well protected in a hostile environment, which is crowded and nutrient-limited. By contrast, sea cucumbers (Holothuroidea), which are soft-bodied echinoderms, and sea hares and nudibranchs (Opisthobranchia), which are mollusks without shells, lack physical protection. Chemical research has substantiated early suggestions that holothurians use triterpenoid glycosides as their defensive agents (*5*). Opisthobranch mollusks, on the other hand, present a more complex picture. For them, virtually all known defensive chemicals are diet-derived. Moreover, herbivores (sea hares) and carnivores (nudibranchs) graze or prey selectively (*6, 7*) and are capable of utilizing for their defense a broad spectrum of molecular entities, which they ingest with their food from algae, sponges, corals, bryozoans, or tunicates. Faulkner and Ghiselin (*8*) have speculated that evolution of mechanisms of chemical defense preceded the loss of shells in dorid nudibranchs (*8*).

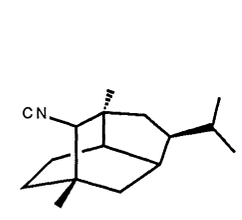
The earliest suggestion of a chemical link between a specific secondary metabolite of the sea hare *Aplysia kurodai* and its diet was made by Irie *et al.* (*9*). They speculated that a 15-carbon isoprenoid, which had previously been described as a constituent of the red alga *Laurencia glandulifera*, might be the precursor of an *Aplysia* metabolite. This suspected chemical connection between molluscan predator and its algal or invertebrate diet has since been confirmed as a result of many investigations of the chemistry of herbivorous (*6*) and carnivorous (*7*) mollusks and their dietary sources. In several instances, some of the ingested chemical substances are not metabolized by the grazer or predator but are stored and used as defensive

agents. An attractive hypothesis, as yet unproven, is that these chemical substances are also responsible for initially attracting the mollusk to its specific dietary source. Nudibranchs have become favored research targets, as these animals feed on different invertebrate phyla and sequester from them organic metabolites ranging from alkaloids to polyacetylenes. They first came to the attention of organic chemists through a metabolite, isocyanopupukeanane (Fig. 1), which bears the rare isocyano function (*10*). It was this report that unambiguously demonstrated a chemical link between a sponge and a nudibranch. Moreover, this research showed that the compound originates in the sponge *Ciocalypta* sp., is retained by the nudibranch *Phyllidia varicosa* in its mantle, and is ichthyotoxic.

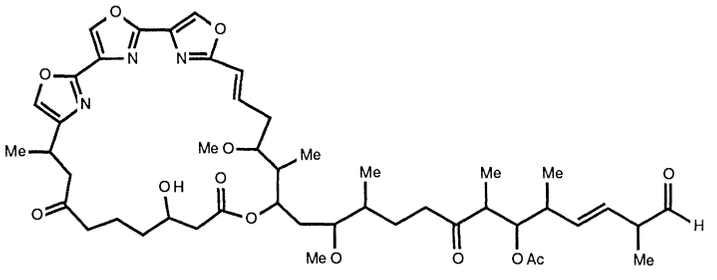
Another, more complex, example of transmission of a protective chemical involves *Hexabranchus sanguineus*, a large (up to 10 by 7 cm), colorful (red and white) nudibranch and a good and graceful swimmer, which has given rise to its trivial name "Spanish dancer." Its egg masses are fluted red ribbons resembling rosebuds (Fig. 2), which are deposited on rocks or corals. These unprotected eggs seem to be free from predation. The bioactive constituents, the ulapualides (*11*) (Fig. 1), have a distinct molecular structure. They are macrolides that encompass three contiguous oxazoles in a 28-membered lactone ring (*12*). One structural feature, a terminal *N*-methylformyl [–N(Me)CHO] group, gives rise to restricted rotation about the amide bond, which prevents ready crystallization and hampers interpretation of nuclear magnetic resonance spectra as six hydrogen and nine carbon atoms close to the *N*-methylformyl terminus generate two signals each in a ratio of 2:1. Because of this complexity, full stereochemical details are still lacking. The compounds exhibit antileukemia and antifungal activity.

The nudibranch *Hexabranchus sanguineus* also contains ulapualides but in far smaller concentration than do the eggs. A dietary source of these compounds is uncertain, although (*13*) a white calcareous sponge, *Leucetta solida*, has been suggested as a possible food source. This seems unlikely because nudibranch and its prey generally display identical or at least compatible pigmentation. Secondary metabolites of *Leucetta* do not contain ulapualides apparently. However, Pawlik *et al.* (*14*) isolated ulapualide-related macrolides from *Hexabranchus* and from a sponge, *Halichondria* sp., collected at the same site in the Marshall Islands. These workers also maintained *Hexabranchus* in an aquarium for 2 months on a *Halichondria* diet and found *Halichondria* spicules in *Hexabranchus* stomachs. Pawlik *et al.* (*14*) further showed in laboratory assays that the macrolides are feeding deterrents when offered with food pellets to a reef fish, *Thalassoma lunare* (wrasse).

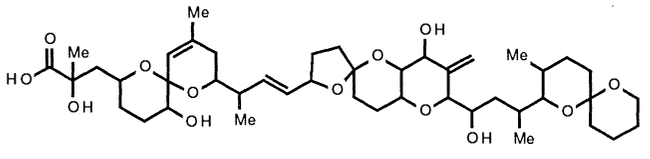
Hexabranchus eggs in Hawaii have one known predator, the cryptically colored aeolid nudibranch *Favorinus japonicus*. No chemical investigation of this animal has been reported. If the aeolid predator accumulates ulapualides, these remarkable compounds



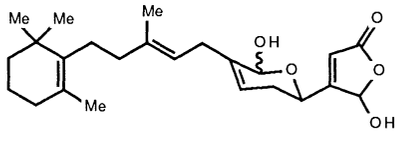
2-Isocyanopupukeanane



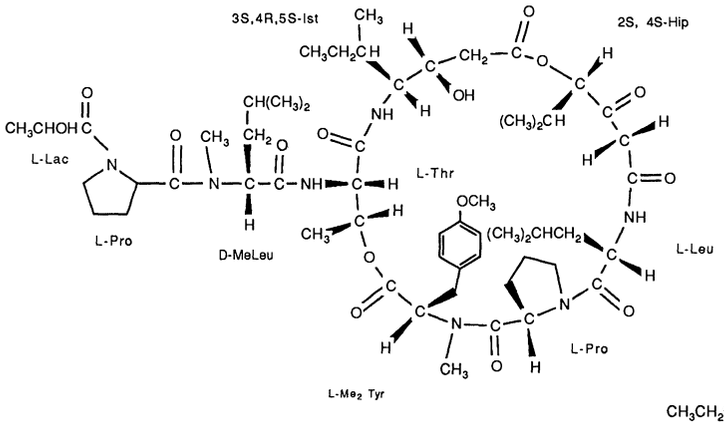
Ulapualide A



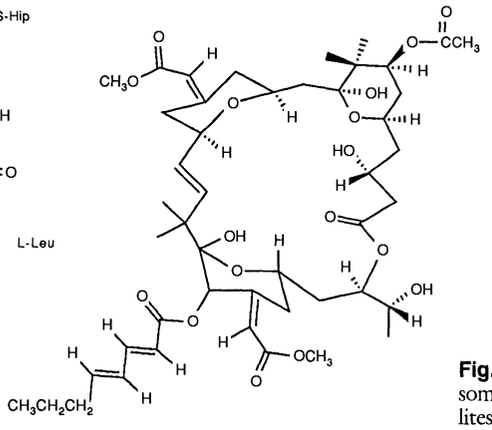
Okadaic acid



Manoalide



Didemnin B



Bryostatin 1

Fig. 1. Molecular structures of some secondary marine metabolites involved in species survival or having biomedical significance.

would provide protection through no less than four trophic levels: sponge to dorid nudibranch to eggs to aeloid nudibranch. Aside from in vitro evidence of antileukemia and antifungal properties, it is not known whether these macrolides will have a biomedical application.

Biomedical Potential

"Drugs from the sea" has been a convenient label for an effort that began in 1960 with a conference entitled "Biochemistry and Pharmacology Derived from Marine Organisms," organized by the New York Academy of Sciences (*15*). Since that time, pharmacological evaluation of secondary marine metabolites has been the sole or at least an implied objective of marine natural product research. Noteworthy achievements of biomedically guided marine exploration include the discovery of the antitumor agents didemnin B and bryostatin 1 (Fig. 1). Bryostatin 1 was first isolated from the bryozoan *Bugula neritina* in 1968 and is currently being evaluated by the National Cancer Institute (NCI) (*16*). Didemnin B, a metabolite of the tunicate *Tridemnum solidum*, was a result of shipboard screening of marine organisms by Rinehart and co-workers (*17*) and is currently in clinical trials at NCI.

Okadaic acid (Fig. 1) is an example of a compound that was first isolated from a sponge, *Halichondria okadai*. Its promising in vitro anticancer activity (at a concentration of 5 ng/ml it inhibited the growth of KB cells by 80 percent) did not carry over to in vivo experiments, and further research was abandoned at that time. After its molecular structure had been elucidated (*18*), okadaic acid was recognized as a metabolite of the marine dinoflagellate *Prorocentrum lima* (*19*). Because of its low concentration (1×10^{-4} percent) in the sponge, okadaic acid had been suspected as the product of a symbiont or an epibiont, but this association was never proven. When the acid was first isolated from *P. lima*, it was found that its chromatographic behavior paralleled that of ciguatoxin. Ciguatoxin is a ciguatera-causing compound that is normally isolated from toxic carnivorous fishes in tropical waters. [Ciguatera is a human illness that is caused by the consumption of ciguateric fishes (*20*), and the molecular structure of the toxin was unknown.] The observation

Fig. 2. Egg masses of the nudibranch *Hexabranchus sanguineus*. [Photograph by B. J. Burreson]

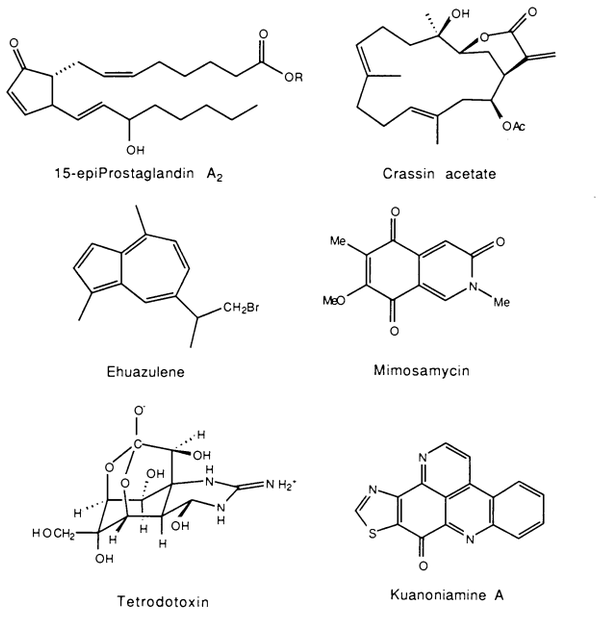


Fig. 3. Molecular structures of some secondary marine metabolites illustrating their organismic origin.

that ciguatoxin and okadaic acid behave similarly during chromatography was confirmation that ciguatoxin belonged to the class of polyether toxins, among them the well-known brevetoxins, that are associated with the organism that is responsible for red tides in the Gulf of Mexico.

A second unexpected property of okadaic acid, unsuspected during its early evaluation, is that of tumor promoter (*21*); however, its mechanism of operation is different from that of such well-studied tumor promoters as the phorbol esters, teleocidin, or aplysiatoxin. The lengthening list of tumor-promoting substances is causing increasing concern in the biomedical community as evidence for environmentally related human cancers accumulates.

Another property of okadaic acid is its inhibition of calcium-activated, phospholipid-dependent protein kinase. The acid was shown to be a powerful inhibitor of protein phosphatase-1 and phosphatase-2A in vitro (*22*). Haystead *et al.* (*23*) further showed that okadaic acid rapidly stimulates protein phosphorylation in intact cells and behaves like a specific protein phosphatase inhibitor in a variety of metabolic processes. They (*23*) also showed that okadaic acid mimics the effect of insulin on glucose transport in adipocytes, a finding that shed new light on lipolysis and fatty acid synthesis.

Another secondary marine metabolite of increasing biomedical significance is manoalide (Fig. 1). Like okadaic acid, this compound was also first isolated from a sponge, *Luffariella variabilis*, collected in the western Caroline Islands (*24*) and was named after Manoa Valley on the island of O'ahu. Structurally it is a sester (25-carbon) terpene, a relatively uncommon class of organic natural products. It has no distinctive structural features except that the molecule has two distinct moieties, one is a hydrocarbon and the other highly oxygenated. The organic extract shows unspectacular antimicrobial activity in vitro against Gram-positive organisms. Detailed pharmacological evaluation of manoalide was carried out by Mayer and Jacobs (*25*). These workers recognized the analgesic and anti-inflammatory properties of manoalide, which led them to investigate its mode of action. Further study showed that manoalide is a potent and irreversible inhibitor of phospholipase A_2. Current research is focusing on delineating the binding site. Mayer and Jacobs have also

shown that manoalide affects the release of arachidonic acid from membrane phospholipids from mouse peritoneal macrophage, which in turn affects eicosanoid release. Manoalide thus may become useful in the treatment of disorders in which eicosanoids are known to participate. The compound is currently under investigation "as a candidate drug for the treatment of skin diseases, where activation of phospholipases and the presence of eicosanoids have been documented" (*25*, p. 140).

The Origin of Secondary Marine Metabolites

Scanning electron micrographs of marine organisms (*26*) show that in the ocean every surface may become a substrate for another micro- or macroscopic organism. Whether these associations are physical, and to what extent chemotaxis is involved, is uncertain. Two examples will illustrate this question.

In contrast to the well-known red tides, which are massive surface blooms of dinoflagellates and which may cause paralytic shellfish poisoning and fish kills in temperate waters, toxic dinoflagellates that cause tropical (that is, ciguatera) fish poisoning are benthic and settle on macroalgae or coral detritus. Although dinoflagellates (*27*) settle selectively, we do not know the mechanism for the selectivity. It is likely to have a chemical basis because the settling organism, *Gambierdiscus toxicus*, produces mixtures of toxins that differ in composition depending on their origin, whether derived from wild or cultured populations. It has not been possible to achieve in the laboratory the same toxin distribution that occurs in wild populations. Ciguatoxin thus originates in a microalga; it settles, probably involving chemotaxis, on a macroalga, which is consumed by herbivorous fishes; these fishes are preyed upon by carnivores, which in turn are eaten by man—along with the toxin unchanged through five trophic levels, a sequence that is without parallel in terrestrial ecosystems.

A second example is found in prostaglandins of marine origin (Fig. 3). Soon after the startling discovery by Weinheimer and Spraggins (*3*) of substantial concentrations of two prostaglandins in a Caribbean gorgonian coral, *Plexaura homomalla*, Corey and co-workers (*28*) showed that a homogenate of the coral will biosynthesize prostaglandins from their customary precursor, arachidonic acid, provided the buffer of the solution is prepared with seawater or with sodium chloride. *Plexaura homomalla* is one of the many species of coelenterates that are associated with symbiotic algae, the zooxanthellae. A preparation of these algae, free from the animal host, did not convert the precursor C_{20} arachidonic acid into prostaglandins (*29*). Fatty acid analysis of the algae revealed that arachidonic acid is only a minor (0.7 percent) component. Hence the algae do not store the prostaglandin precursor, but they conceivably could synthesize it and supply it to the coral, a sequence that would be difficult to prove experimentally.

Research on marine invertebrate prostaglandins, the punaglandins (*30*), reinforces the above interpretation that the prostaglandins are synthesized by the animal rather than the alga. The punaglandins have been isolated from a coral, *Telesto riisei*, that is devoid of symbiotic algae.

The process is different in other organisms and other environments. In a Caribbean gorgonian, *Pseudoplexaura porosa*, whose principal secondary metabolite is a di-(C_{20}) terpene with a cembrane skeleton (crassin acetate, Fig. 3), the situation is reversed. Papastephanou and Anderson (*31*) showed that terpene synthesis in *P. porosa* occurs in a cell-free homogenate of the zooxanthellae rather than in the host animal.

These concepts have been demonstrated by examination of sessile marine invertebrates in deep water (below 100 m), where no photosynthetic algae exist. The secondary metabolites, sesqui-(C_{15}) and di-(C_{20}) terpenes as well as nitrogenous pigments of deep-sea gorgonians and zoantharian coelenterates are structurally closely related to constituents of comparable shallow-water species. For example, Fusetani *et al.* (*32*) had isolated the brilliant blue sesqui-(C_{15}) terpene guaiazulene from the shallow-water gorgonian *Euplexaura erecta*. Guaiazulene is a compound that was known from terrestrial essential oils, where it is most likely an artifact produced during distillation of nonaromatic precursors. Subsequently Li and Scheuer (*33*) described from a deep-water (350 m) gorgonian, family Paramuriceidae, a series of halogen-substituted guaiazulenes. The most significant of these compounds is ehuazulene (Fig. 3), which is chiral, an indication that introduction of the halogen (bromine) atom cannot be a random event but is enzymatically controlled in the animal. A dietary source of this and other deep-sea metabolites would seem unlikely because an animal that is firmly anchored to its substrate on the bottom of the ocean has no food choices.

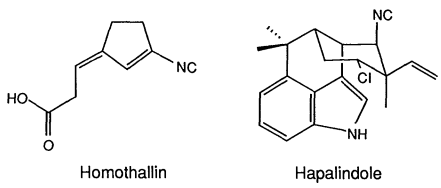


Fig. 4. Molecular structures of two isocyano compounds whose biosynthesis has been studied. See also 2-isocyanopupukeanane in Fig. 1.

The foregoing examples are among a small number of cases in which the specific organism producing an isolated and described chemical substance has been identified. There are other instances for which the evidence is circumstantial. In the course of an investigation of a nudibranch predator and its sponge prey, Gulavita (*34*) isolated a series of isoquinolinequinones, among them the known antibiotic mimosamycin (Fig. 3). The sponge *Xestospongia* sp. was found not to contain this metabolite, but it was found in the mollusk *Jorunna funebris*. Other workers (*35*), however, had isolated mimosamycin from a sponge, *Reniera* sp. The compound was originally described by Fukami *et al.* (*36*) as having been derived from the terrestrial *Streptomyces lavendulae* no. 314. Isoquinolinequinones constitute a small but well-recognized group of terrestrial antibiotics (*37*). The producer of mimosamycin in marine invertebrates might be a *Streptomyces* sp. and not the nudibranch. It should be possible to collect these two marine invertebrates again and attempt to isolate, culture, and identify any epi- or symbiont living with the sponge or the nudibranch. As a final step, one would have to assess the natural product profile of the cultured microorganisms.

Another line of circumstantial evidence is that widely disparate biota seemingly produce identical secondary metabolites. The best-known example is that of the puffer fish toxin, tetrodotoxin (Fig. 3), which has been reported from puffers, salamanders, an octopus, crabs, and a red alga (*38*). A suspected epiphytic origin of tetrodotoxin was buttressed by the failure on the part of several workers to incorporate labeled tetrodoxin precursors in puffers or newts. Eventually Yasumoto and co-workers (*38*) succeeded in culturing a bacterium, *Alteromonas* sp., isolated from an alga, *Jania* sp., and in identifying tetrodotoxin and a derivative in the culture broth. In addition, they isolated the same two compounds from a *Pseudomonas* sp. that was found in the skin of the puffer *Fugu poecilonotus* (*39*). The wide distribution of tetrodotoxin in marine bacteria was underlined in a study by Simidu *et al.* (*40*), who identified the toxin in marine bacteria of the genera *Vibrio*, *Aeromonas*, and *Alteromonas*. Possibly at variance with these results is a report by Kodama *et al.* (*41*), who identified tetrodotoxin in poison-secreting glands in the skin of several species of puffer fishes, *Takifugu* spp.

An as yet unresolved example of the isolation of closely related

metabolites from distant phyla has been demonstrated by isolation of several pentacyclic alkaloids from a tunicate and its prosobranch mollusk predator, among them kuanoniamine A (Fig. 3) (*42*). Closely related compounds had been reported from other tunicates but also from a sea anemone (Coelenterata) and a sponge. Sponges are the most primitive multicellular marine invertebrates, whereas tunicates are chordates, which are phyletically close to vertebrates.

Biosynthesis of Marine Metabolites

In the preceding section I cited evidence that the locus of prostaglandin biosynthesis is the invertebrate animal rather than the algal symbiont. Because of the importance of prostaglandins in biomedicine, the mechanism of the biosynthesis in marine invertebrates is also of interest. Is it identical with the well-studied pathway for mammalian prostaglandins, or does it differ? Corey and coworkers (*43*) have shown conclusively that the two mechanisms do indeed differ. Whereas the key mammalian biosynthetic intermediate is the 9,11-endoperoxide, in *Plexaura homomalla* and other Caribbean gorgonians it is the pentadienyl-8,9-epoxide.

An unsolved question is biosynthesis concerns the precursors of the isocyano (NC) function, which is well known from the laboratory but is rare in living systems. The question was raised soon after the first naturally occurring isocyano compound was isolated from a terrestrial microorganism (*44*). It was shown that the nitrogen atom of the isocyano function was derived from the amino acid, which furnished the carbon framework of the antibiotic, but the source of the isocyano carbon atom remained unknown. As additional isocyano antibiotics were described from terrestrial sources, it became evident that amino acids, either directly or indirectly as in the homothallins (Fig. 4) (*45*), provided the carbon skeleton and the nitrogen atom, but no source could be traced for the isocyano carbon. A different situation obtains in isocyano-bearing metabolites from marine organisms, principally sponges and nudibranchs. These compounds have an isoprenoid rather than an amino acid–derived carbon skeleton. Hence the biosynthetic question that had to be addressed was that of the source of both carbon and nitrogen atoms of the isocyano function.

As discussed above, the first marine molluscan isocyano metabolite was isolated from the nudibranch *Phyllidia varicosa* and was traced to its food source, the sponge *Ciocalypta* sp. (*10*). The compound had a sesqui-(C_{15}) terpenoid skeleton (Fig. 1). In fact, virtually all known marine isocyano compounds are terpenes. Hapalindole (Fig. 4) is of terrestrial microalgal origin and an exception, as it is constructed of amino acid and isoprenoid moieties (*46*). Bornemann *et al.* showed that cyanide is an efficient precursor of the isocyano group. Carbon and nitrogen atoms of cyanide were also utilized for isocyano biosynthesis in the sponge *Ciocalypta* sp. (*47*), but no source of cyanide in the marine environment has been identified. A search for unconventional amino acids in *Ciocalypta* sp. proved fruitless (*48*). The biosynthetic pathway of the terpene skeleton of these compounds is equally unknown.

By comparison with terrestrial higher and lower plants, biosynthetic methodology in marine invertebrates is undeveloped. Early experiments were carried out on live sponges in situ (*49*) with the use of stable as well as radioactive isotopes. Sponges metabolize rather slowly, and many species do not survive longer than a week in an aquarium. Relocation of the animal and its substrate to a convenient inshore location has been successful (*50*). However, as in all in situ experiments, changing climatic conditions and predation cause difficulties. Preparation of cell-free extracts has recently been successful (*51*). This technique will allow experimentation under controlled conditions. The next step in improving the methodology would be isolation of the enzymes that are involved in biosynthesis.

REFERENCES AND NOTES

1. W. Bergmann and D. C. Burke, *J. Org. Chem.* **20**, 1501 (1955).
2. Y. Hashimoto and T. Okaichi, *Ann. N.Y. Acad. Sci.* **90**, 667 (1960).
3. A. J. Weinheimer and R. L. Spraggins, *Tetrahedron Lett.* (1969), p. 5185.
4. F. J. Schmitz, in *Marine Natural Products*, P. J. Scheuer, Ed. (Academic Press, New York, 1978), vol. 1, pp. 241–297.
5. D. J. Burnell and J. W. ApSimon, in *Marine Natural Products*, P. J. Scheuer, Ed. (Academic Press, New York, 1983), vol. 5, pp. 287–389.
6. P. J. Scheuer, *Naturwissenschaften* **69**, 528 (1982).
7. P. Karuso, in *Bioorganic Marine Chemistry*, P. J. Scheuer, Ed. (Springer-Verlag, Berlin, 1987), vol. 1, pp. 31–60.
8. D. J. Faulkner and M. T. Ghiselin, *Mar. Ecol. Prog. Ser.* **13**, 295 (1983).
9. T. Irie *et al.*, *Tetrahedron Lett.* (1965), p. 3619.
10. B. J. Burreson, P. J. Scheuer, J. Finer, J. Clardy, *J. Am. Chem. Soc.* **97**, 4763 (1975).
11. The name ulapualide (in Hawaiian, ula means red and pua means flower) was coined because of the appearance of the egg masses.
12. J. A. Roesener and P. J. Scheuer, *J. Am. Chem. Soc.* **108**, 846 (1986).
13. E. A. Kay, *Hawaiian Marine Shells* (Bishop Museum Press, Honolulu, 1979), p. 471.
14. J. R. Pawlik, M. R. Kernan, T. F. Molinski, M. K. Harper, D. J. Faulkner, *J. Exp. Mar. Biol. Ecol.* **119**, 99 (1988).
15. *Ann. N.Y. Acad. Sci.* **90**, 615–950 (1960).
16. M. Suffness, D. J. Newman, K. Snader, in *Bioorganic Marine Chemistry*, P. J. Scheuer, Ed. (Springer-Verlag, Berlin, 1989), vol. 3, pp. 131–168.
17. K. L. Rinehart, Jr., *et al.*, *Science* **212**, 933 (1981).
18. K. Tachibana *et al.*, *J. Am. Chem. Soc.* **103**, 2469 (1981).
19. Y. Murakami, Y. Oshima, T. Yasumoto, *Bull. Jpn. Soc. Sci. Fish.* **48**, 69 (1982).
20. P. J. Scheuer, in *Mycotoxins and Phycotoxins 88*, S. Natori, K. Hashimoto, Y. Ueno, Eds. (Elsevier, Amsterdam, 1989), pp. 265–276.
21. M. Suganuma *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1768 (1988).
22. C. Bialojan and A. Takai, *Biochem. J.* **256**, 283 (1988).
23. T. A. J. Haystead *et al.*, *Nature* **337**, 78 (1989).
24. E. D. deSilva and P. J. Scheuer, *Tetrahedron Lett.* **21**, 1611 (1980).
25. A. M. S. Mayer and R. S. Jacobs, in *Biomedical Importance of Marine Organisms*, D. G. Fautin, Ed. (California Academy of Sciences, San Francisco, 1988), pp. 133–142.
26. J. M. Sieburth, *Microbial Seascapes* (University Park Press, Baltimore, 1975).
27. Y. Shimizu *et al.*, *Bull. Jpn. Soc. Sci. Fish.* **48**, 811 (1982).
28. E. J. Corey, W. N. Washburn, J. C. Chen, *J. Am. Chem. Soc.* **95**, 2054 (1973).
29. ———, *ibid.* **96**, 934 (1974).
30. B. J. Baker, R. K. Okuda, P. T. K. Yu, P. J. Scheuer, *ibid.* **107**, 2976 (1985).
31. C. Papastephanou and D. G. Anderson, *Comp. Biochem. Physiol. B* **73**, 617 (1982).
32. N. Fusetani, S. Matsunaga, S. Konosu, *Experientia* **37**, 680 (1981).
33. M. K. W. Li and P. J. Scheuer, *Tetrahedron Lett.* **25**, 587 (1984).
34. N. K. Gulavita, thesis, University of Hawaii at Manoa, Honolulu (1987).
35. J. M. Frincke and D. J. Faulkner, *J. Am. Chem. Soc.* **104**, 265 (1982).
36. H. Fukami *et al.*, *Tetrahedron Lett.* (1977), p. 3825.
37. T. Arai and A. Kubo, in *The Alkaloids, Chemistry and Pharmacology*, A. Brossi, Ed. (Academic Press, New York, 1983), vol. 21, pp. 55–100.
38. T. Yasumoto, M. Yotsu, A. Endo, M. Murata, H. Naoki, *Pure Appl. Chem.* **61**, 505 (1989).
39. M. Yotsu *et al.*, *Toxicon* **25**, 225 (1987).
40. U. Simidu, T. Noguchi, D.-F. Hwang, Y. Shida, R. Hashimoto, *Appl. Environ. Microbiol.* **53**, 1714 (1987).
41. M. Kodama *et al.*, *Toxicon* **24**, 819 (1986).
42. A. R. Carroll and P. J. Scheuer, in preparation.
43. E. J. Corey, S. P. T. Matsuda, R. Nagata, M. B. Cleaver, *Tetrahederon Lett.* **29**, 2555 (1988) and references therein.
44. M. S. Edenborough and R. B. Herbert, *Nat. Prod. Rep.* **5**, 229 (1988).
45. J. E. Baldwin *et al.*, *Tetrahedron* **41**, 1931 (1985).
46. V. Bornemann, G. M. L. Patterson, R. E. Moore, *J. Am. Chem. Soc.* **110**, 2339 (1988).
47. P. Karuso and P. J. Scheuer, *J. Org. Chem.* **54**, 2092 (1989).
48. A. R. Carroll and P. J. Scheuer, in preparation.
49. M. R. Hagadone, P. J. Scheuer, A. Holm, *J. Am. Chem. Soc.* **106**, 2447 (1984).
50. M. J. Garson, *J. Chem. Soc. Chem. Commun.* (1986), p. 35.
51. R. Herb and P. J. Scheuer, in preparation.