

Exploring the Ocean—Stating the Case for Chemistry

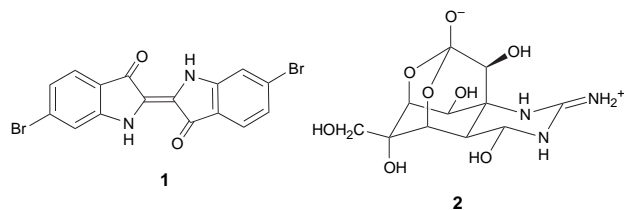
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“Chemistry Is Everywhere” was the theme of the American Chemical Society’s National Chemistry Week several years ago. Those who live on large continents tend to forget that the earth is the blue planet, dominated by water without which there would be no life as we know it. The dramatic view of the earth with the Hawaiian islands as the hub (Fig. 1) illustrates the ocean’s dominance, particularly in Hawai’i, an isolated archipelago with no major body of land closer than 3,000 km. Yet for most of recorded history the resources of the ocean remained largely undiscovered except for fish, a few invertebrates, seaweed, sand, and sodium chloride. Even after exploration of the ocean became a scientific endeavor, chemistry per se was confined to sea water analysis. As recently as 1987 at a conference on the chemistry of tropical marine systems in Brazil, all of carbon chemistry was dealt with in two categories, “dissolved” and “particulate” carbon.

Marine Natural Products: Early Examples

There were notable exceptions to this biased attitude on the part of chemical oceanographers. Harvesting of *Murex* spp. and related marine snails for the production of the prized dye Tyrian purple dates back several thousand years and is perhaps the earliest example of marine biotechnology (1). More compelling stimuli to the study of carbon chemistry were occasional incidents in which food harvested from the sea caused sickness or even death. Halstead’s treatment of “marine zootoxology” (2) traces encounters with harmful marine organisms to their earliest records. However, a firm bond between marine toxins and organic chemistry was established rather slowly. While Friedländer deduced the correct structure of Tyrian purple (1) in 1909 (3), the structure of tetrodotoxin (2), the toxic principle of the pufferfish, was first disclosed in 1964 (4); it represents a major benchmark in the study of marine natural products.



Complexity of molecular structure and availability of the organism explain the 55-year hiatus. Neither event, however, created much of a stir among organic chemists. Tetrodotoxin was merely yet another esoteric molecule of interest to a few academics. The Japanese people had long ago learned how to cope with the hazards of “fugu” and its potential consequences.

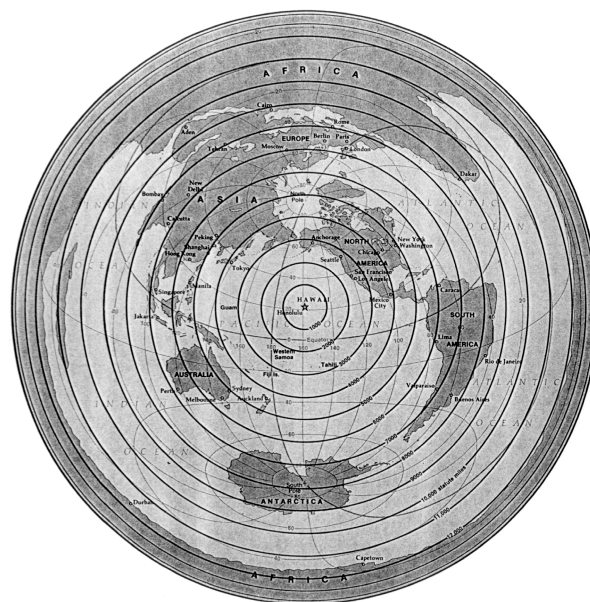


Figure 1. Planet Earth with Hawai’i at its center.

Mainstream organic chemistry in the 1960s was excited over theoretical concepts and the revolution in instrumentation. Pharmaceutical companies were satisfied with the huge success of the penicillin legacy, a fortuitous marriage of microbiology and fermentation technology. The 1969 communication by Weinheimer and Spraggins (5) more than any other event began to send organic chemists to the sea. At a time when interest in the physiological properties of the prostaglandin hormones was high and their availability from natural sources or by synthesis was minuscule, isolation of respectable quantities of these compounds from an obscure marine invertebrate was a stellar event, indeed.

The search for additional sources of marine prostaglandins was disappointing. It was many years before new organisms and new structures were discovered¹ (6), and by that time organic chemists had perfected a number of elegant and efficient syntheses. However, the notion of searching for new and perhaps unique carbon compounds in the ocean had taken hold. This awareness was enhanced by the publication in 1973 of the first book whose title, *Chemistry of Marine Natural Products*, firmly linked organic chemistry with marine science (4). The frontispiece of the book revealed a wealth of marine biota, which had virtually been mare incognitum except to marine biologists. This illustration (Fig. 2) soon became and has remained a familiar feature in oral presentations

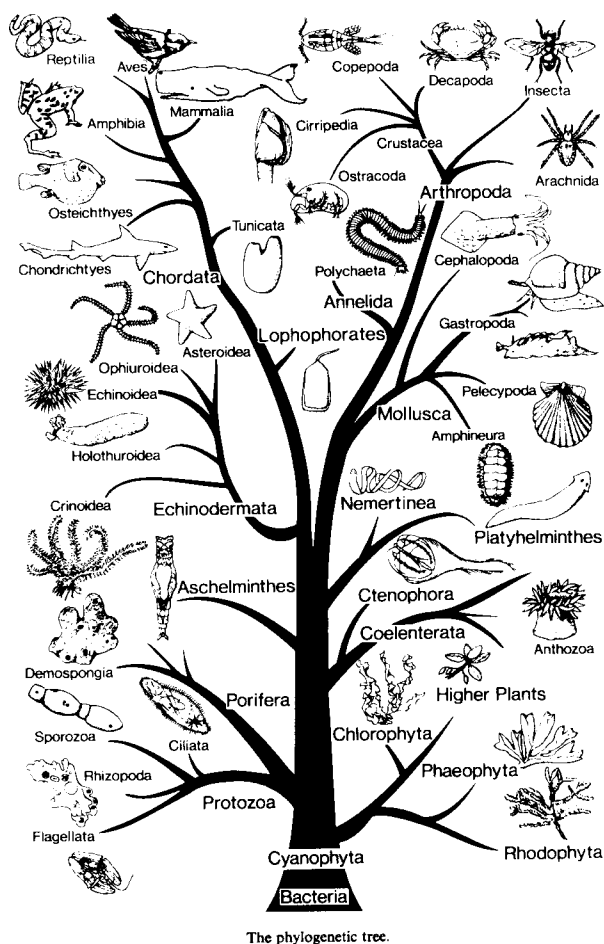
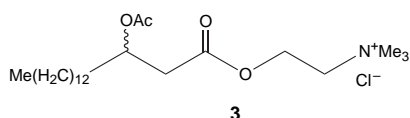
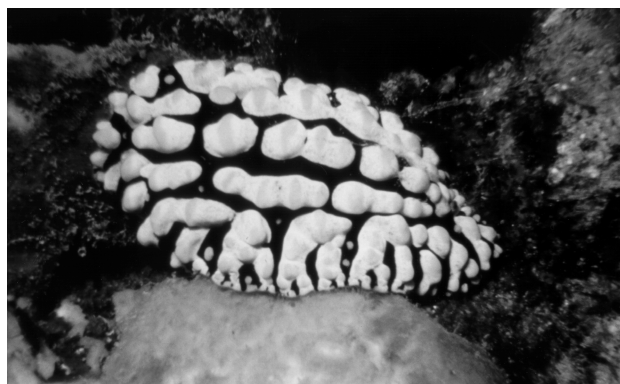


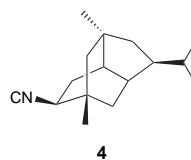
Figure 2. A phyletic tree of marine biota.

worldwide. It has served as a convenient guide to much of our marine research. In this paper I will attempt to show with examples from my laboratory how even relatively modest coral reefs (the Hawaiian islands are young as well as remote) can generate a wide spectrum of molecular structures and illuminate previously unknown biological phenomena.

My fascination with the budding field had been aroused by my friendship with a number of marine biologist colleagues at the University of Hawaii who early-on steered my research toward ecological and biomedical topics (7–9). An early project for example, was based on an observation by an ichthyologist that a small slow-swimming reef fish, the boxfish, *Ostracion lentiginosus* (Fig. 3), when stressed, releases a toxin that kills other aquarium fishes without their gasping (which is commonly associated with dying fish) (10). We succeeded in identifying a skin secretion, pahutoxin, as the choline ester of β -acetoxy palmitic acid (3). It was the first chemically identified interspecific marine pheromone (kairomone) (11). My former student Art Goldberg showed in a subsequent cooperative study with Australian colleagues that soaplike defensive skin secretions are a general property of trunkfishes (Ostraciidae) (12).

Figure 3. *Ostracion lentiginosus* (boxfish).Figure 4. *Phyllidia varicosa* (nudibranch).

Discovery of an invertebrate kairomone, 9-isocyanopupukeanane (4), also was triggered by an observation by then graduate student Bob Johannes (13) that the nudibranch (a carnivorous gastropod mollusk) *Phyllidia varicosa* possesses a skin secretion that is toxic to fish and crustaceans. Our earliest studies revealed that when confined to an aquarium the animals soon cease secretion of the kairomone. The biological literature was silent on the diet of the animals. Searching the Pupukea reef and tidepools for the prey of the animals eventually led us to a sponge, *Ciocalypa* sp. (originally believed to be *Hymeniacidon* sp.) and a reliable source of 9-isocyanopupukeanane (Fig. 4) (14). Ever since, nudibranchs and their prey—sponges, tunicates, other mollusks—have been favorite targets of marine natural product research. And sponges, thanks to their accessibility and size, are almost everybody's favorite research object.



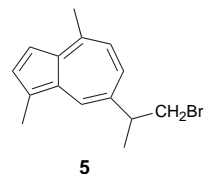
Fish Poisoning Leads to Research by Submersible

Search for bioactive marine metabolites became a strong component of my research program through my participation in a multidisciplinary team that investigated ciguatera fish poisoning (8, 15). This is a rarely fatal but often disabling human intoxication caused by the ingestion of tropical reef fishes. Its wide variability in time and place provided a major mystery unmatched by the other two well-known marine food toxins—tetrodotoxin (see above), which is largely confined to a single genus of fishes, and the red tide toxins (saxitoxin, brevetoxin), which announce themselves by a dramatically colored ocean surface and which were traced long ago to the accumulation of toxic unicellular algae, various species of dinoflagellates. During the years when marine scientists were searching for a source organism of ciguatera toxin, we eagerly pursued any lead no matter how tenuous. Among many others we checked out an oral report (16) that a deep-sea gorgonian coral, *Gerardia* sp., causes severe skin irritation to those who handle it.

Gorgonian or horny corals have a flexible skeleton of organic polymer. By contrast, hard or stony corals have a rigid frame of calcium carbonate and are the foundation of coral reefs. Soft corals share the physiology of the other corals, but lack a skeleton. Gorgonian corals, because of their flexibility, can be fashioned into prized jewelry, an activity going back to prehistoric times (17). In 1958, SCUBA divers discovered a bed of black coral off the island of Maui; some ten years later jewelry-quality gorgonian corals were discovered at a depth of 400 m off the southeast coast of O'ahu by employing a minisubmersible.

By 1971 The University of Hawai'i, via its Sea Grant College Program, became involved in mapping and describing the precious corals and advising the industry on harvesting and conservation. Eventually, the university established its own submersible operation, which was accessible to all qualified investigators. Through the Hawai'i Undersea Research Laboratory my coworkers and I were privileged to dive to the ocean floor, first aboard the two-person (the pilot and a scientist) Makali'i, which subsequently was replaced by the larger Pisces V. After a slow 20-minute descent, past the photic zone, passing an occasional fish or squid, landing in total darkness on the ocean floor with a slight bump, there was truly revealed to me a new dimension of life on earth. The huge treelike formations of gold, red, and blue coral anchored in the ocean

bottom were an unforgettable sight unattainable by description or even photograph (Fig. 5) Equally spectacular were the spiraling whiplike bioluminescent bamboo corals (Fig. 6), made even more dramatic when the floodlights of the Makali'i were turned off and the light continued to travel back and forth over the length of the animal. Contrasting with the visual feast, the chemistry of the deep sea gorgonians proved to be plebeian. A chiral bromoazulene, ehuazulene (5), was a welcome exception (18).



Molluscan Chemistry—a Lead to Marine Biology

Immediate access to deep ocean waters is a consequence of the volcanic origin of the Hawaiian islands, which have risen (and continue to rise) vertically from the ocean floor. Their latitude, between 19° and 28° North (affording comfortable water and air temperatures throughout the year), and their small land areas provide an unparalleled opportunity to carry out marine research that calls for frequent and ready access to the same habitats. Our successful investigation into the nudibranch–sponge chemical relationship described earlier was made possible by being within an hour's drive of Pupukeya Bay on the north shore of O'ahu. The rocky shore is characterized by tidepools, lava tubes, and small underwater caves. During one of our field collections in the late 1970s, Gary Schulte, at the time a graduate student, collected some specimens of a cephalaspidean mollusk, subsequently identified as *Philineopsis speciosa* (Fig. 7). According to conventional wisdom at the time, secondary metabolites of marine mollusks would be of polypropionate biosynthetic origin. In fact, another graduate student, Stephen Coval, who inherited the project, did isolate two such compounds in addition to an alkylpyridine (19, 20). However, this proved to be merely the overture to what has become a major opus. In the course of his research, Steve Coval had observed that a polar extract of *P. speciosa* contained a peptide; this was a pregnant observation, indeed, which over the years has spawned a large family of peptides. The initial compound

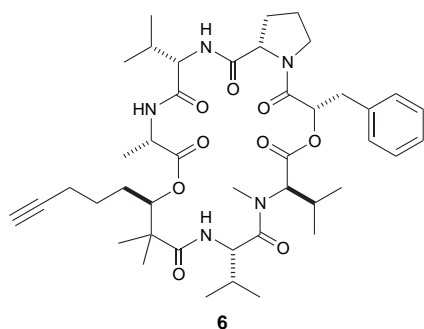


Figure 5. *Corallium* sp. (gold coral).

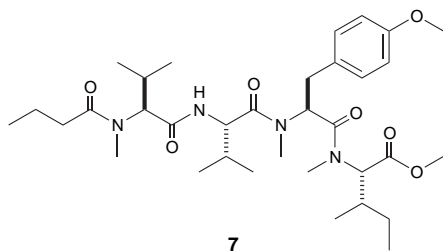


Figure 6. Isididae family (bamboo coral).

kulolide² (**6**) (20–22), so named because of its long gestation period, is a prime example of a research project that requires proximity of the ocean to a sophisticated laboratory.



There were several good reasons for the protracted research. *P. speciosa* is found only during midsummer nights with an incoming tide, when the animals emerge from the sand to feed and mate. The population density over the years has varied from but a few animals for several years, followed by virtual gluts. Furthermore, during the early years of the research, interpretation of the NMR spectra of kulolide was hampered by the existence of two conformers with many overlapping signals at the prevailing field strength of 300 MHz. Reisolation of kulolide (**6**) from the bountiful collections of 1994 and 1995 and careful chromatography produced five additional cyclic depsipeptides, which differed from kulolide and from each other predominantly in their hydroxy acids (unpublished data from this laboratory). A sixth peptide, the acyclic pupukeamide (**7**), provided a clue to the possible origin of kulolide (**6**), which had been a mystery to us throughout the years (23).



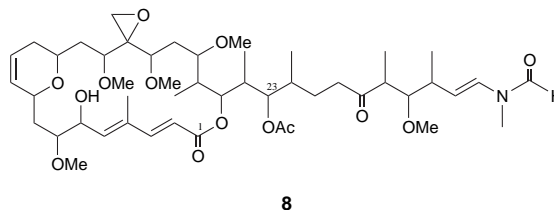
The amino acid makeup of pupukeamide instantly reminded us of the majusculamides, which Moore and coworkers had isolated from the cyanophyte (blue-green alga) *Lyngbya majuscula* (24, 25). Since *P. speciosa* is a carnivore, the linear peptide could not come directly from a plant. There had to be one or several missing links. Cimino's closely related work with Mediterranean mollusks in the same family, Aglajidae, in which he had traced the polypropionate constituents of *Aglaja depicta* to its prey, *Bulla striata* (26), appeared to offer a clue; but we rarely encountered *Bulla* spp. specimens at Pupukea. We did see the sea hares *Stylocheilus longicaudus* and *Dolabella auricularia*. We had dismissed their potential role because their chemistry (the aplysiatoxins [27] and dolastatins [28]) bore no resemblance to kulolide. But as soon as pupukeamide was characterized, it all became clear. The cyanophyte *L. majuscula*, a known dietary source for *Stylocheilus*, was the missing link! Laboratory feeding experiments readily confirmed that *Philineopsis* will accept *Stylocheilus* as well as *Dolabella* as food. In summary, *P. speciosa* had revealed to us hitherto hidden



Figure 7. *Philineopsis speciosa* (mollusk).

ecological relationships in addition to ten metabolites: two polypropionates, one alkylpyridine, six cyclic and one acyclic peptide—no doubt a remarkable record for any sea slug!

Discovery of yet another *Philineopsis* constituent, 23-acetoxytolytoxin (**8**), an acetate of the known macrolide (29), came about in unexpected fashion. We had asked scientists at the University's Cancer Research Center to assay the *Philineopsis* peptides. They were suspicious when, despite the peptides' structural similarity, only one compound showed characteristic activity in an assay involving rat fibroblast cells. Even more significantly, the researchers were reminded of the results they had obtained with the macrolide tolytoxin (30). Rigorous rechromatography of the suspect fraction successfully separated 23-acetoxytolytoxin (**8**). The bioassay results had been caused by a 0.3% contamination with acetoxytolytoxin.



Where does tolytoxin originate? It is a metabolite of a cyanophyte that was first collected from a shed on Fanning Island. The accumulating evidence that ultimately all *Philineopsis* metabolites are produced by cyanophytes is bolstered by the presence in the kulolides of *N*-methyl amino acids, which are normally associated with algal metabolites.

Species diversity on coral reefs is often favorably compared to the rich diversity found in tropical rain forests. I have been fortunate to have hiked in the Hawaiian rain forest under the guidance of the late eminent botanist Harold St. John (7) and to have dived at a few coral reefs. As a visual experience the ocean is unmatched, replete with brilliant color, breathtaking shapes, and constant movement. The rain forest is a tangled green jungle with only an occasional bright flower. Only a veteran explorer is able to spot and trace an individual plant species. In both ecosystems new species await description. There is little doubt that many more marine biota and their intricate ecological roles await discovery. Perhaps the unfolding fascinating chemistry of the marine ecosystem

will have fortunate by-products by reviving much needed scholarship in organismic marine biology and by highlighting the importance of conservation and management of marine resources.

Acknowledgments

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Notes

1. The cover photograph of the November 1994 issue of *J. Chem. Educ.* depicts a coral that produces marine prostaglandins.
2. *Kulo* is a Hawaiian word for *slow*.

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