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MARINE NATURAL PRODUCTS RESEARCH: A LOOK INTO THE DIVE BAG[1]

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ABSTRACT.—The study of natural products has again become appreciated as one of the principal forces that shape organic chemistry. The virtually untapped wealth of marine biota has added a new dimension to natural product research, while confirming the enduring elements of the world of organic chemistry: molecular architecture, biosynthesis, function, and application.

The beautifully pigmented spines and shells of sea urchins (phylum Echinodermata) provided my first non-culinary encounter with marine life. It was in the early 1950s (1) and a time when natural products of flowering plants were my principal research objectives. A growing fascination with marine biota gradually steered my research interest from the rainforest to the ocean, where I discovered a wealth of molecular diversity and biological significance (2). I will illustrate this theme with examples from previous and recent researches.

The uncomplicated chemistry of echinoderm pigments, predominantly naphthoquinones, was a good match for the primitive state of separation and structural elucidation techniques available in the 1950s. A modest literature on sea urchin pigments existed, but it was characterized by confusion rather than rigor. Many of the so-called spinochromes and echinochromes proved to be mixtures of closely related compounds. Chang (3) devised a chromatographic technique which allowed separation of unambiguously pure compounds and thus paved the way for meaningful comparison of closely related and/or identical compounds bearing a bewildering alphabet soup of suffixes. Luckily, it was a time when nmr instruments and mass spectrometers became commercially available. This enabled us to establish a firm structural base of naphthoquinone chemistry.

Of profound importance for my subsequent research interests was my participation, from 1957 on, in a multidisciplinary study of ciguatera fish poisoning (4). In contrast to the well-understood red tides of the temperate zones that cause so-called shellfish poisoning, or of the Gulf of Mexico red tides which lead to massive fish kills, or of puffer fish poisoning, which affects a single family of food fish in Japan (5), ciguatera fish poisoning was a problem that apparently was associated with tropical coral reefs; it was little known or appreciated by the marine research community before World War II. I will not repeat my recent historical account (4) of ciguatera research. Instead, I will detail one facet of this complex collage, which has had ramifications in many areas of biomedical science: the story of okadaic acid.

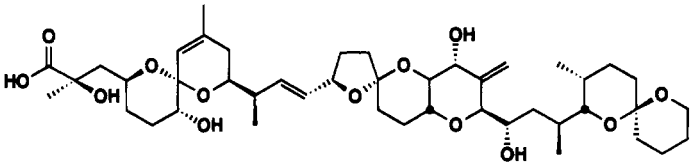
It began in 1975, seven years before anyone suspected a relationship between okadaic acid and ciguatera. At the Marine Natural Products Symposium in Aberdeen, Scotland, Dr. Y. Tsukitani of the Fujisawa Pharmaceutical Company told me of a compound, halichondrine A, which he and his colleagues had isolated from a sponge, *Halichondria okadai*, collected on the Pacific coast of Japan. In preliminary in vitro bioassays the compound displayed impressive anti-KB activity, but subsequent in vivo data were disappointing. Hence Fujisawa was dropping the project and I was offered the compound for structure determination, which I readily accepted. Parenthetically, it was the only

[1]The 1994 American Society of Pharmacognosy Research Achievement Award Address delivered at the International Congress on Natural Products Research, Halifax, Nova Scotia, Canada, 4 August 1994.

time when one of our projects began with a crystalline (ca. 90% pure) metabolite. Recrystallization from MeOH and a name change[2] to okadaic acid were all that was needed to begin structural studies (6). A crystalline *o*-bromobenzyl ester became the entry to a molecular structure [**1**], which had also been secured concurrently by Schmitz and co-workers at the University of Oklahoma on a sample isolated from a Caribbean *H. melanodocia* (7). The 1980 Marine Natural Products Symposium in Brussels, Belgium, provided the venue for the disclosure, a mutual surprise for the Oklahoma and Hawaii groups.

How is all of this related to ciguatera? A twenty-year search for the originating organism, believed to be a fine filamentous alga, had ended in 1977 with Yasumoto's discovery of the toxic dinoflagellate *Gambierdiscus toxicus* (8). The two principal toxic fractions, containing the lipid-soluble ciguatoxin and the H_2O-soluble maitotoxin, could be isolated from wild populations, but the difficult laboratory culture of a benthic alga averse to bright light and to agitation produced only maitotoxin. Hence the search for other ciguatoxin-producing strains of *G. toxicus* and for other microalgae continued. Examination of a unicellular culture of the dinoflagellate *Prorocentrum lima* revealed two Et_2O-soluble toxins, one of which proved to be identical with okadaic acid [**1**] (10,11), the structure of which had recently been disclosed (7). The startling observation that okadaic acid [**1**] and ciguatoxin behaved alike on tlc was the first and vital clue that ciguatoxin and okadaic acid belonged to the same structural type of polyethers derived from long-chain fatty acids. It would be nearly ten more years before the molecular structure of the extremely scarce ciguatoxin [**2**] could be secured (12–14). Once the structural parallel was established, Hokama utilized okadaic acid as a convenient and more readily available prototype for development of a diagnostic test for ciguatoxin (15). The close structural resemblance between okadaic acid and the diarrhetic shellfish toxins, caused by the dinoflagellate *Dinophysis fortii*, provided yet another link among the growing number of seafood toxins (13).

Other, seemingly unrelated properties of okadaic acid gradually came to light and



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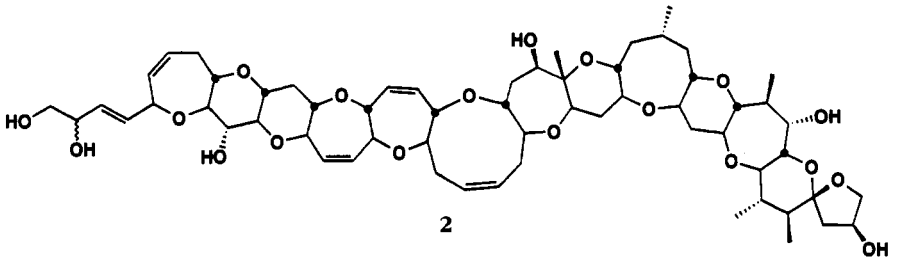

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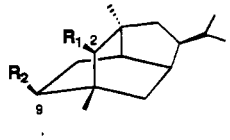
[2]Trivial names of natural products should not be misleading; they should reflect the chemical nature of the particular compound.

overshadowed its transient role in the structural elucidation of ciguatoxin. Fujiki and co-workers, who have long studied the mechanism of tumor promotion, found that okadaic acid acts as a non-traditional (i.e., non-phorbol ester) tumor promoter (16). Independent of tumor promotion research, okadaic acid has become a widely used molecular probe because of its selective inhibition of phosphatases 1 and 2A (17,18). It has become an article of commerce.

The overture to the foregoing opus, which played for more than 30 years was a public health concern that eventually led to a valuable tool in biomedical research. The illustration that follows describes our involvement in coral reef ecology. It began with an observation by a marine biologist (19) that a nudibranch (*Phyllidia varicosa*, a carnivorous shell-less gastropod mollusk) secretes a milky fluid lethal to fish and crustaceans. Our decision to probe the chemical nature of this defensive secretion led us to its dietary source, unknown at the time, a sponge, *Ciocalypta* sp., and enabled us to elucidate the structures of the defensive agents, the isocyanopupukeananes [**3**] (20); moreover, it led to an enduring interest in cyano-isocyano chemistry (21) and above all, to a continuing exploration of the marine fauna of Pupukea Bay on the north shore of O'ahu. Our success prompted many other workers to explore nudibranch chemistry (22).

Cephalaspideans (characterized by a head shield and frequently shelled) constitute another order of carnivorous gastropod mollusks. Some feed on other cephalaspideans (23). A chance collection of some specimens of a cephalaspidean, *Philinopsis speciosa*, made while snorkeling at Pupukea, was the beginning of a 15-year liaison with this animal. During much of the year (September to April) high surf precludes any collecting activity. Even during the summer months the animal emerges from its sandy burrows and appears in the Pupukea tidepools predominantly during moonless nights with an incoming tide. Our early collections yielded representatives of the anticipated polypropionates, niuhinones A [**4**] and B [**5**] (24) and pulo'upone [**6**] (25). Subsequent collections revealed a cyclic depsipeptide, kulolide, which was difficult to characterize as it existed in two conformations in varying proportions depending on temperature and solvent (26,27).

Among herbivorous mollusks, the Anaspidea, which include the sea hares, have been studied extensively. A less well-known order, the Sacoglossa, are capable of ingesting functioning chloroplasts from their algal diet. A few known secondary metabolites are unexceptional polypropionates (28). Some sacoglossans of the genus *Elysia* are reported



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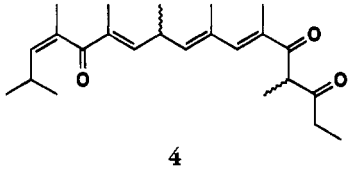
R_1=NC, R_2=H
R_2=NC, R_1=H



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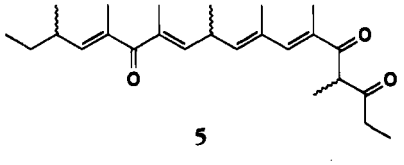

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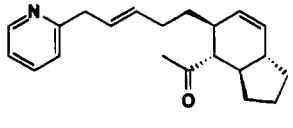

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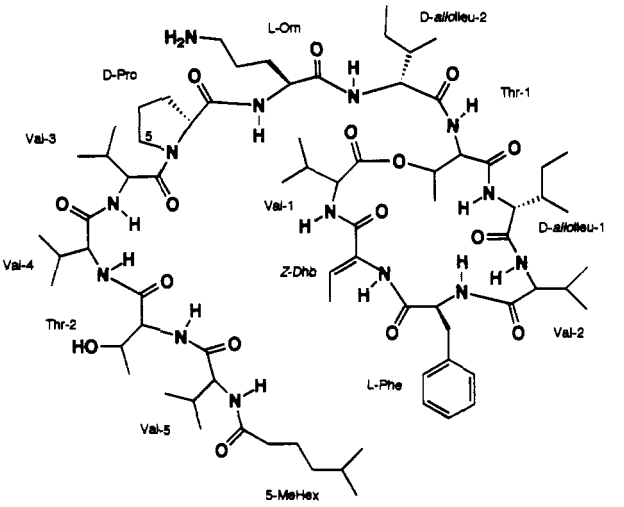
TABLE 1. Comparative Composition Data of *Elysia* Peptides.

	Kahalalide		Amino Acids														Fatty Acid
	Molecular Formula	Mol Wt	Ala	Arg	Dhb	Gly	Ileu	Leu	Orn	Phe	Pro	Ser	Thr	Trp	Tyr	Val	
A	$C_{46}H_{67}N_7O_{11}$	894.1						2		2		1	2				2-MeBu
B	$C_{45}H_{63}N_7O_{11}$	878.0				1		1		1	1	1	1		1		5-MeHex
C	$C_{47}H_{63}N_9O_{10}$	914.1		1			1			1			1		2		Bu
D	$C_{31}H_{45}N_7O_5$	595.7		1							1			1			7-Me-3-Octol
E	$C_{45}H_{69}N_7O_8$	836.1	2					2			1			1			9-Me-3-Decol
F	$C_{75}H_{124}N_{14}O_{16}$	1477.9			1		2		1	1	1		2			5	5-MeHex

to feed on calcareous algae, among them the genera *Udotea* and *Halimeda* (29), from which Paul has isolated diterpenes (30). No food source was reported for *Elysia rufescens*, which we collected at Black Point on the south shore of O'ahu, but by analogy with other *Elysia* spp. we anticipated terpenoid secondary metabolites. We found in fact no trace of terpenoids, but a wealth of depsipeptides, the kahalalides, ranging from a C_{31} tri- to a C_{75} tridecapeptide (Table 1). Except for a single amino acid, (*Z*)-2-aminobut-2-enoic acid, in kahalalide F [**7**], all other residues are of common occurrence. The fatty acids are largely obscure and uncomplicated (31,32). Kahalalide F [**7**] exhibits selective activity against solid tumors as well as antiviral activity (33). While collecting *E. rufescens*, we observed that the animal grazes on a green alga, *Bryopsis* sp. Extraction of the alga yielded the acyclic analogues, kahalalides G [**8**] and F [**7**], the latter in a lower concentration than in *Elysia*.

Although mollusks are convenient concentrators of secondary metabolites, many of them are small and often return to shallow water only to spawn and to feed. Most sponges, on the other hand, are reasonably large and sessile, hence readily collected. While some presumed sponge metabolites are in fact the products of algal or bacterial symbionts or epibionts, sponges are capable of synthesizing secondary metabolites and have been a prolific source of new molecular architecture, often coupled with desirable biological activity. The first notable modern example was Bergmann's isolation of three new nucleosides from a Caribbean sponge, *Cryptotethia crypta* (34–37). Two of the nucleosides contained the uncommon sugar arabinose in place of ribose, which is a universal constituent of primary metabolism. The antiviral drug Ara-A, a nucleoside that eventually arose from those early isolates is, as the name implies, an arabinoside (38). After nearly 40 years of exploration of sponge chemistry, new combinations of sugars and purine bases remain to be discovered. From an Indonesian sponge, *Theonella* sp., we recently isolated a nucleoside kumusine[3], which encompasses a chlorinated purine base and an alkylsugar (T. Ichiba and Y. Nakao, unpublished data from this laboratory).

Another recent example of a secondary metabolite from a sponge with structural features resembling primary metabolites is *Spongia oceania*, which we collected near Kihei, Maui. One of its metabolites is a phosphate ester, an essential part of nucleotides



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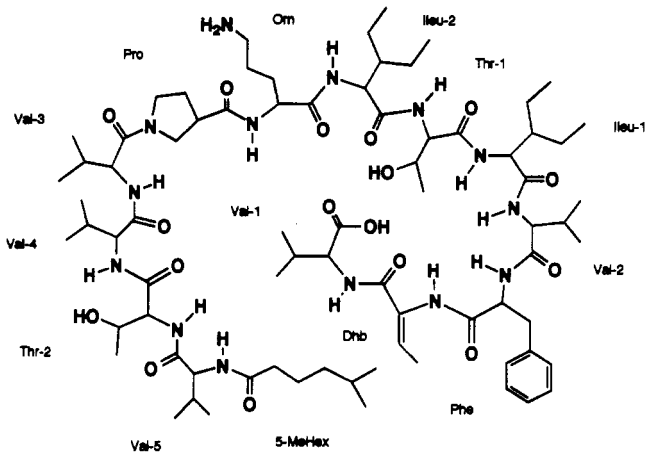
[3]*Kumu*, Hawaiian for branch of a tree, describes the tree-like morphology of the sponge, *Theonella* sp.



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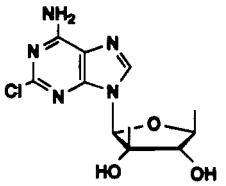
but rare in secondary metabolites. The compound, pokepola ester [**10**] is phosphoric acid esterified with 5-methylhexanol and homoserine. In turn, the amino group of homoserine forms an amide with a trisnorsesquiterpene (39). This phosphate ester exhibits no antitumor activity, while the corresponding free alcohol, pu'elamide[4] [**11**] is selectively active against solid tumors[5] (J.A. Palermo and L.A. Tungpalan[6], unpublished data from this laboratory).

Hawaiian waters are virtually devoid of shallow-water gorgonians (order Gorgonacea, subclass Octocorallia, class Anthozoa, phylum Coelenterata) (40). In 1976, we collected



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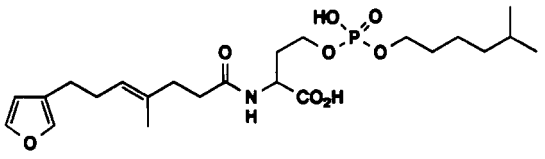

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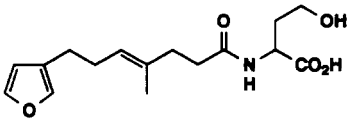

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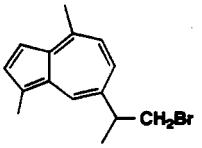

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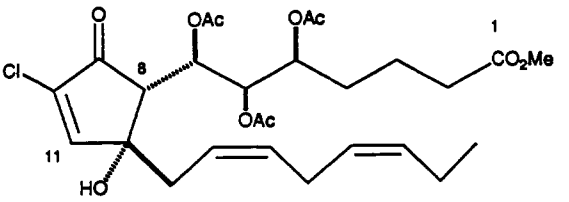
[4]*Spongia oceania* is black; the name is derived from *pu'ele* (Hawaiian)=black.

[5]Activity determined at Wayne State University, Detroit, Michigan, by the NCI-funded National Cooperative Drug Discovery Groups program.

[6]MBRS undergraduate participant.

TABLE 2. Structural Features of the Punaglandins.



Compound	C-12 alcohol	C-12 acetate	$\Delta^{17,18}$	17,18-Dihydro	$\Delta^{7,8}$	Other
1	●		●			
2		●	●			
3	●			●		
4		●		●		
5	●		●		●	
6		●	●		●	
7	●			●	●	
8		●		●	●	
9	●		●		Z	
10		●	●		Z	
11	●			●	Z	
12		●		●	Z	
13	●		●		●	10,11-O
14	●			●	●	10,11-O
15	●		●			7-H
16		●	●			7-H
17	●			●		7-H
18	●		●			$\Delta^{5,6}$
19	●			●		$\Delta^{5,6}$

at Enewetak, Marshall Islands, an animal that appeared to be a gorgonian. We referred to it as "the white gorgonian" until it was identified as an octocoral of the order Telestacea, *Telesto riisei*. From this collection we isolated two pregnanes (41) with a ring A dienone. *T. riisei* is a member of the fouling community and was first observed in Hawaiian waters in Pearl Harbor in 1974 (40). Our first Hawaii collection was from Pupukea on the north shore of O'ahu. Subsequently, we routinely collected the animal in murky water at the entrance of the Ala Wai Yacht Harbor. We never again found any pregnanes or other steroidal compounds. Instead, we isolated no fewer than 19 related prostanoids, the punaglandins, which have a common structural feature, an α-chlorocyclopentenone moiety (Table 2) (42–44).

In contrast to the dearth of gorgonians inside the reef, the deep waters beyond the reef are rich in gorgonians and readily accessible by minisubmersible: 93 species of gorgonians have been described from deep Hawaiian waters (45). From a beautiful blue animal, a member of the family Paramuriceidae, which we collected at a depth of 350 m by the minisubmersible *Makali'i*, we isolated a number of azulenic pigments, among them the chiral ehuazulene [**12**] (46). Aside from the chirality of the compound its most remarkable property is its blue color, which has no counterpart in terrestrial natural pigments, where blue coloration is rare and usually the result of a molecular complex rather than the expression of a single chromophore.

Tyrian purple, the ancient dye extracted from *Murex* spp. and related mollusks, was perhaps the first commercial marine natural product (47). The bright and varied colors

of a coral reef first enticed me to study the chemistry of marine life. As we have seen, beautiful colors have continued their attraction, but also have led to questions of function, of origin, and of possible utility in the terrestrial ecosystem.

ACKNOWLEDGMENTS

Only a few of my talented and enthusiastic co-workers are mentioned herein. I owe all of them a debt of gratitude for having supported and guided me on this exciting watery journey. I am also grateful for steadfast financial support from the National Science Foundation; the Sea Grant College Program (NOAA); PharmaMar, S.A.; and the National Cooperative Drug Discovery Groups program of the National Cancer Institute.

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