The Chemistry and Chemical Ecology of Octocorals (Coelenterata, Anthozoa, Octocorallia)

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I. Introduction

A. General

If indeed it is true that all life originated in the oceans, some organisms remained in the sea, others escaped onto land, while yet others soared on the winds. All have captured our imagination, and now in a retroevolutionary sense, chemists are turning their gaze back from the land to their watery origins. This has become possible with the advent of scuba diving equipment and deep-sea submersible collection facilities. The recent developments in chromatographic techniques and the widespread use of high-field NMR spectrometers have meant that complete structural elucidation is now possible on small amounts of difficulty won material. Because of these developments, chemists from Oklahoma, Illinois, and Arizona, together with colleagues on the east and west coasts of North America, in Hawaii, New Zealand, and Australia, and in Okinawa and New Caledonia, to single out a few marine research centers, have all found excitement and adventure in the discovery of the secrets of organisms hidden beneath the sea.[1]

Although the oceans cover almost two-thirds of the Earth's surface, living creatures in the sea constitute only 2% of the organic material therein.[2] The majority of living biomass exists closely associated with man and the pollution-impacted fringes of the continents and habitated islands. It turns out that most of the oceans are virtual deserts; coral reefs and atolls constitute rare oases of life, often far removed from the mainland.[3] Marine life is most rich and varied in tropical waters, and coral reefs provide the major life support systems for this diverse biota. Coral reefs are produced by the efforts of countless numbers of coral polyps and coralline algae which transform soluble calcium and bicarbonate ions in sea water into limestone.[4] Together they provide solid, robust surfaces on which marine life can aggregate. Chemistry is in action at every step in the process, as I hope this review will reveal. Two main areas of the globe possess coral reefs: the tropical Western Atlantic and the Indo-Pacific regions.[3] The

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John Coll was born in Sydney, Australia, and was educated at the University of Sydney, completing his B.Sc. degree with First Class Honours in Organic Chemistry in 1966. He pursued Ph.D. research with Charles Shoppee and Ruth Gall (nee Lack) on the mechanisms of nucleophilic substitutions of 19-nor cholesterol derivatives, being awarded his Ph.D. from the University of Sydney in 1969. At the University of Illinois with Nelson Leonard, he successfully completed the synthesis of "manxane and manxine", novel bicyclo-[3.3.3]undecanes, and then took up a lectureship at Imperial College, London, with Derek Barton. Research in London again involved synthesis and mechanistic studies of steroid systems. In 1972, he returned to Australia to a lectureship in Organic Chemistry at James Cook University in Townsville. Over the last 19 years, he led a research group investigating the natural products chemistry (with Bruce Bowden) and chemical ecology (with Paul Sammarco) of soft corals. He has spent sabbaticals with Pierre Potier at Gif sur Yvette, France, with John Faulkner and Bill Fenical at Scripps Institution of Oceanography, and with Bob Pettit at Arizona State University, and has recently taken the post of Pro-Vice-Chancellor at the University of Central Queensland, in Rockhampton, but still beside the Great Barrier Reef, Australia.
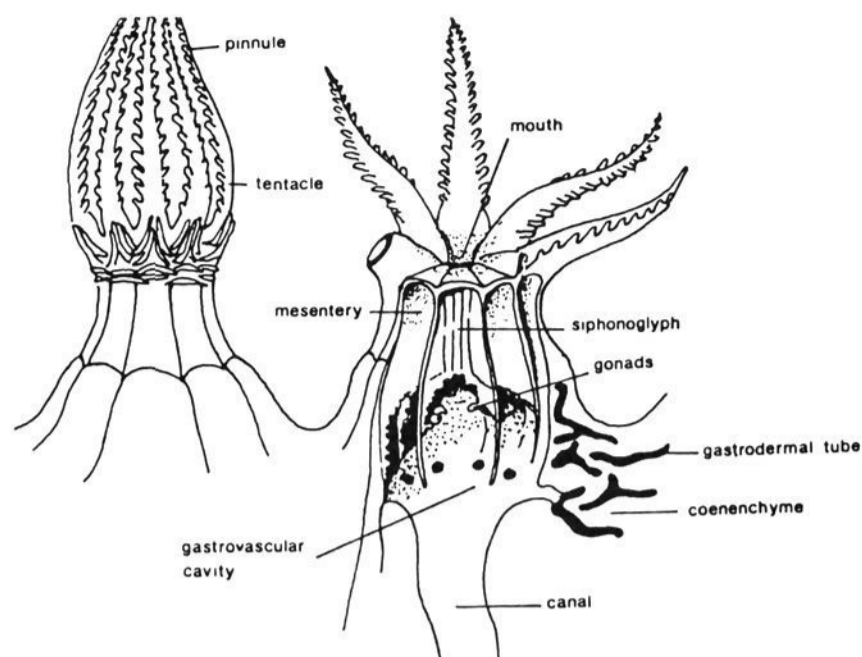
Figure 1. Schematic diagram illustrating the essential features of octocoral polyps. (Line drawing: S. La Barre, adapted from Moore's *Invertebrate Zoology*.)

former, from the chemical prospection point of view, centers on the Caribbean, while the latter focuses on the Great Barrier Reef, Australia, and the islands of the South Pacific and Okinawa in the north.

B. Corals, Octocorals, and Coral Reefs

The polyp is the basic unit of a coral colony: some (e.g. the "slipper coral" *Fungia* sp.) are solitary, single-polyp corals. The majority of corals, however, are colonial marine invertebrates belonging to the phylum Coelenterata (Cnidaria), class Anthozoa. The polyp is essentially a tube which may be linked to other polyps in the same colony by a system of canals leading from the base of each polyp. The oral opening possesses a ring of tentacles used for food capture; the organisms discussed in this review have eight pinnate tentacles (class Octocorallia) (Figure 1), while the more familiar hard or scleractinian corals (class Hexacorallia) possess multiples of six tentacles.[3,4] Nutrition in corals thus derives from the ingestion of zooplankton (i.e. copepods, invertebrate larvae, phytoplankton, etc.).[5] There is however a second source of energy and carbon. Most corals exist in a symbiosis with dinoflagellate algae called zooxanthellae. These endo-symbionts belong to what is probably a multispecies aggregate called *Symbiodynium microadriaticum*[6] and carry out photosynthesis, providing energy for coral calcification which drives the reef building process of hard and soft corals alike. Hard or scleractinian corals produce beautifully sculptured aragonitic (similar to limestone) homes for their polyps ($\sim$95% of total colony is $CaCO_3$). The

polyps can withdraw completely into these exoskeletons to avoid predation. They have little need for chemical defense and indeed are relatively uninteresting in their chemical composition. Soft corals and many gorgonian corals also secrete calcitic (similar to limestone) skeletal particles which are imbedded in a fleshy matrix called coenenchyme (0–75% of total colony is $CaCO_3$). These spicules or sclerites, as they are called, are usually rather small (0.1–1 mm). Occasionally however, they may be larger (5–10 mm) and play a distinctly defensive role for the polyp (Figure 2a, *Nephthea* sp.). They help to reinforce the canal walls and are one of the major characters used in taxonomy of the group[7] (Figure 2b, *Sinularia* sp.). Most gorgonian octocorals also have a central rodlike axis made of gorgonin, a matrix similar in structure and properties to that found in finger nails or avian beaks. This proteinaceous matrix is often calcified.

Although octocorals are not strictly regarded as hermatypic or reef building corals, evidence is accumulating which suggests that "spiculite"—an aggregation of soft coral spicules produced by *Sinularia* species—may provide reinforcement for many Indo-Pacific reefs.[8] J. C. Lewis has even suggested that soft corals may form most of the framework of certain types of Western Pacific and Indian Ocean reefs.[9]

C. Octocoral Taxonomy

Taxonomically, the Octocorallia comprise six orders (Table I): the Alcyonacea (fleshy soft corals), Telestacea (common fouling organisms), Gorgonacea (horny or gorgonian soft corals), Stolonifera (matlike or stolon-borne soft corals), Pennatulacea (sea pens), and Coenothecalea (blue-stony corals). Chemistry has been reported or noted from all six orders. The Alcyonacea and Stolonifera are prominent in the Indo-Pacific, while the Gorgonacea and Pennatulacea dominate the Caribbean. There is little crossover of species between the two regions.

D. Octocorals and Chemists

Clearly octocorals did not attract chemists' attention because they produced limestone. The excitement was

(a)
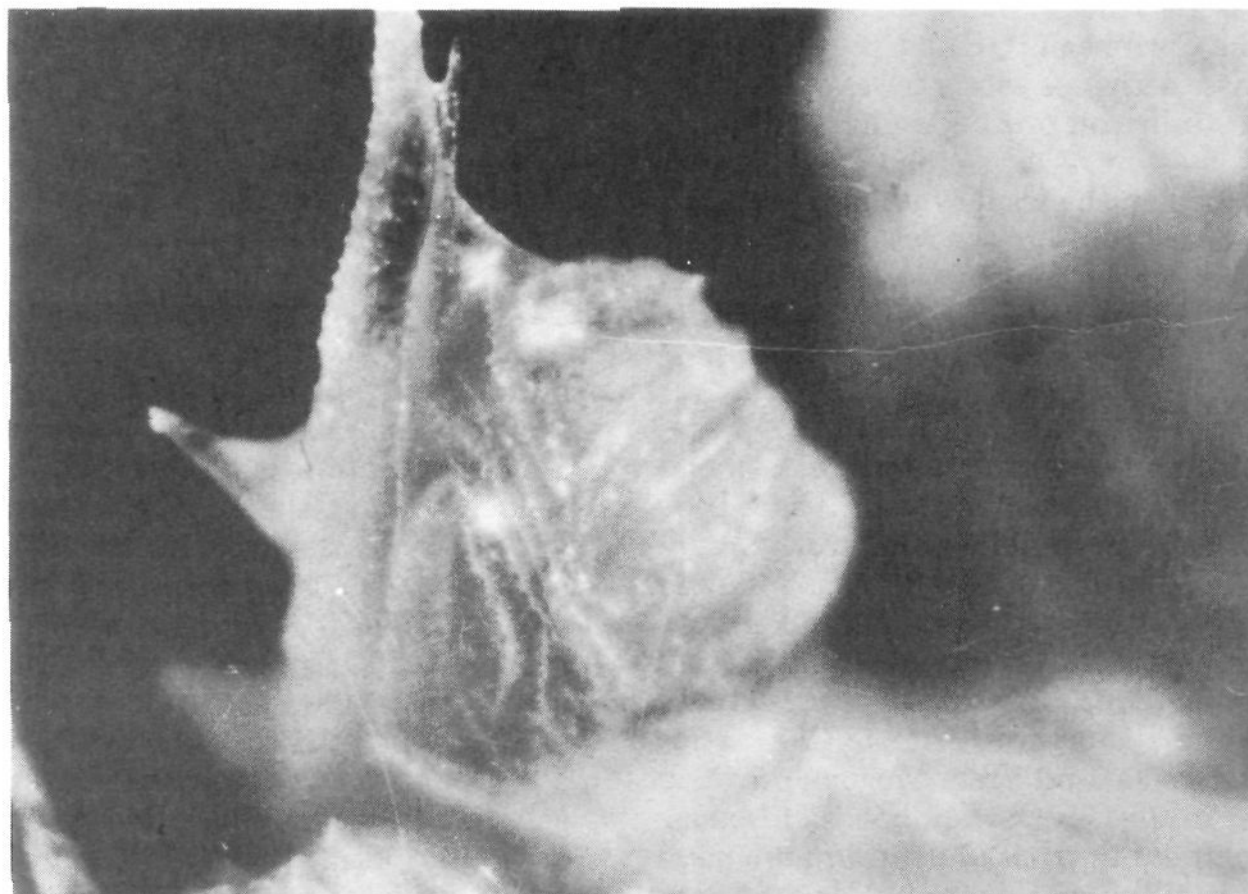
(b)

Figure 2. Physical protection afforded to alcyonacean octocorals by limestone spicules: (a) *Nephthea* species with protected polyps and (b) *Sinularia* species with large spicules packed throughout the basal coenenchyme.

TABLE I. Major Taxonomic Relationships within the Coelenterata (Cnidaria)

phylum	subphylum	class	order	common name
Coelenterata (Cnidaria)	Medusozoa	Hydrozoa		
		Scyphozoa		
		Cubozoa		
	Anthozoa	Ceriantipatharia		
		Alcyonaria (Octocorallia)	Alcyonacea	soft corals
			Gorgonacea	
			Pennatulacea	
			Stolonifera	
			Telestacea	
			Coenothecalea	hard corals
		Zoantharia (Hexacorallia)	Scleractinia	
			Actiniaria	
			Corallimorpharia	
			Zoanthiniaria (Zoanthidae)	

in the range of secondary metabolites contained in their tissues. Bergmann[10] carried out extensive studies on the chemistry of corals and other marine invertebrates in the 1940s and 1950s, and one of Bergmann's students, Leon Ciereszko, moved on to a faculty position at the University of Oklahoma at Norman. He started to look at gorgonians from the Caribbean and Gulf of Mexico. He was attracted by the odors of gorgonians and started to look at their terpenoid chemistry.[11] In 1968, Weinheimer and Spraggins, following up on some of Ciereszko's chemical leads, reported the isolation of the prostaglandin (15R)-PGA$_2$ from *Plexaura homomalla*.[12] It constituted almost 1–2% of the dry weight of the gorgonian, and the quest for "Drugs from the Sea" was on in earnest. Coupled with the chemical studies of gorgonians, a major ecological study was

undertaken to assess the viability of "farming *P. homomalla*" in the Caribbean.[13] E. J. Corey short circuited many of these activities with a series of chemical and biosynthetic studies on prostaglandin synthesis[14] and biosynthesis.[15]

The coral (rather than the algal symbiont) certainly makes prostaglandins,[16] but Corey showed that chemists could make them even better than corals.[14] It was against this background that I arrived in Townsville in 1972 and took my first dive on the Great Barrier Reef.

E. Literature Base and Scope of the Review

The foundations of marine natural products chemistry and the literature published before 1973 have been reviewed by Paul Scheuer in his seminal work *Chemistry of Marine Natural Products*.[1] He has followed this important work with two series which review the evolution of the science to the present: *Marine Natural Products: Chemical and Biological Perspectives*, Volumes I-V,[1][7] and *Bio-organic Marine Chemistry*, Volumes 1-3.[18] Joe Baker and Vreni Murphy provided two early volumes in the C.R.C. Handbooks of Marine Science series *Compounds from Marine Organisms*,[19] which tabulate structural and pharamacological data up to 1974, and D. John Faulkner has provided a comprehensive literature review of marine natural products chemistry (omitting only sterols and common lipids) since 1977 in regular reviews which appear in *Natural Products Reports*.[20] A 1977 article[21] provided a background of the earlier literature and set the stage for his more recent reports. The later reviews also provide details of the synthesis of marine natural products, and Faulkner has available a computer data base incorporating the information in his reviews.[22]

Against this background of extensive literature coverage, it would be pointless to try to update Faulkner's 1991 review or to write an encyclopedic review of octocoral chemistry. I have chosen instead to provide a review of the natural products chemistry of octocorals as it impinges on their biology and ecology.

II. Octocoral Chemistry

A. Chemotaxonomy

1. Early Approaches

Terpenoid chemistry predominates across the Octocorallia.[23] It was the presence of volatile sesquiterpenes in the Gorgonacea which initially attracted Leon Ciereszko to this group;[11] it was the subsequent isolation of prostaglandins by his colleagues at Oklahoma which made them important targets for investigation,[12] but it has been with sesquiterpenes and particularly diterpenes that the octocoral literature has expanded.[24]

Gerhart showed that there was chemotaxonomic value associated with the distribution of terpenoid types across the Gorgonacea.[25] He found that using the methods of numerical taxonomy he was able to achieve association of species into genera and families which correlated favorably with classical spicule-based taxonomy.[7] A similar approach is clearly called for within the Alcyonacea.

The first practical use of chemical characters as an aid to taxonomic discrimination was work reported by Kashman[26] in which he used GC-derived fingerprints of sesquiterpene distribution for distinguishing between closely related species. Arguments against routine use of chemistry to define species is the considerable seasonal variation in chemical content within the same species[27] and the fact that the same cembrane may occur in different families (e.g. Nephtheidae and Alcyoniidae) of soft corals.[28] These considerations do not however rule out the molecular taxonomy approach which is much broader, and its application to the Alcyonacea is in progress.[29]

2. Stereochemical Aspects

In 1978, Tursch et al.[24] surveyed the known distribution of terpenoids across the octocorallia. It seemed that the cembranoids in the Gorgonacea usually had the 1R configuration while the alcyonacean equivalents tended to have the 1S configuration. Although this appears to be the predominant situation, at least one exception is now known. Thunbergol from *Sinularia facile* and *Lobophytum pauciflorum* (Alcyonacea) has been shown to have the opposite configuration to that predicted.[30] The second point which seemed general in 1978[24] was that certain genera produced only sesquiterpenes or only diterpenes. In two cases these generalizations have now broken down. *Xenia novaebritannia*[31] and *Nephthea chabroli*[32] each contained both sesquiterpenes and diterpenes. *Xenia* and *Nephthea* had previously been reported to contain only diterpenes. While this is still a useful generalization, it is no longer the rule.

3. Terpene Distribution across the Alcyonacea

Table II summarizes the terpene classes and principal carbon skeletons (see Schemes I and II) derived from each genus of alcyonacean octocorals. These are similar to the character set used by Gerhart[25] for his cladistic analysis of the Gorgonacea based on chemistry. From Table II for the Alcyonacea (true soft corals), of which there are three large multigeneric families, the Alcyoniidae, Nephtheidae, and Xeniidae, the following generalizations about the nature and distribution of diterpenes within genera and families can be deduced:

1. In the family Alcyoniidae

(a) *Sinularia* and *Sarcophyton* spp.: 100% of their diterpenoid metablites are based on the cembrane nucleus (structure types I-VI; see Scheme I).

(b) *Lobophytum* spp.: 70% of their diterpenoid metabolites are based on the cembrane nucleus. The remaining 30% are all from the germacrene group (structure types VII-X; see Scheme I).

(c) *Cladiella* spp.: 100% of the diterpenes are based on cladiellane (i.e. structure type V, i.e. a cyclized cembrane).

(d) *Parerythropodium* spp.: Only sesquiterpenes are produced.

2. In the family Nephtheiidae

(a) *Nephthea* spp.: They contain sesquiterpenes and/or diterpenes (70% of which are based on cembrane).

(b) *Litophyton* spp.: 100% cembranoid diterpenes are reported.

(c) *Lemnalia, Paralemnalia, and Capnella* spp.: They contain only sesquiterpenes.
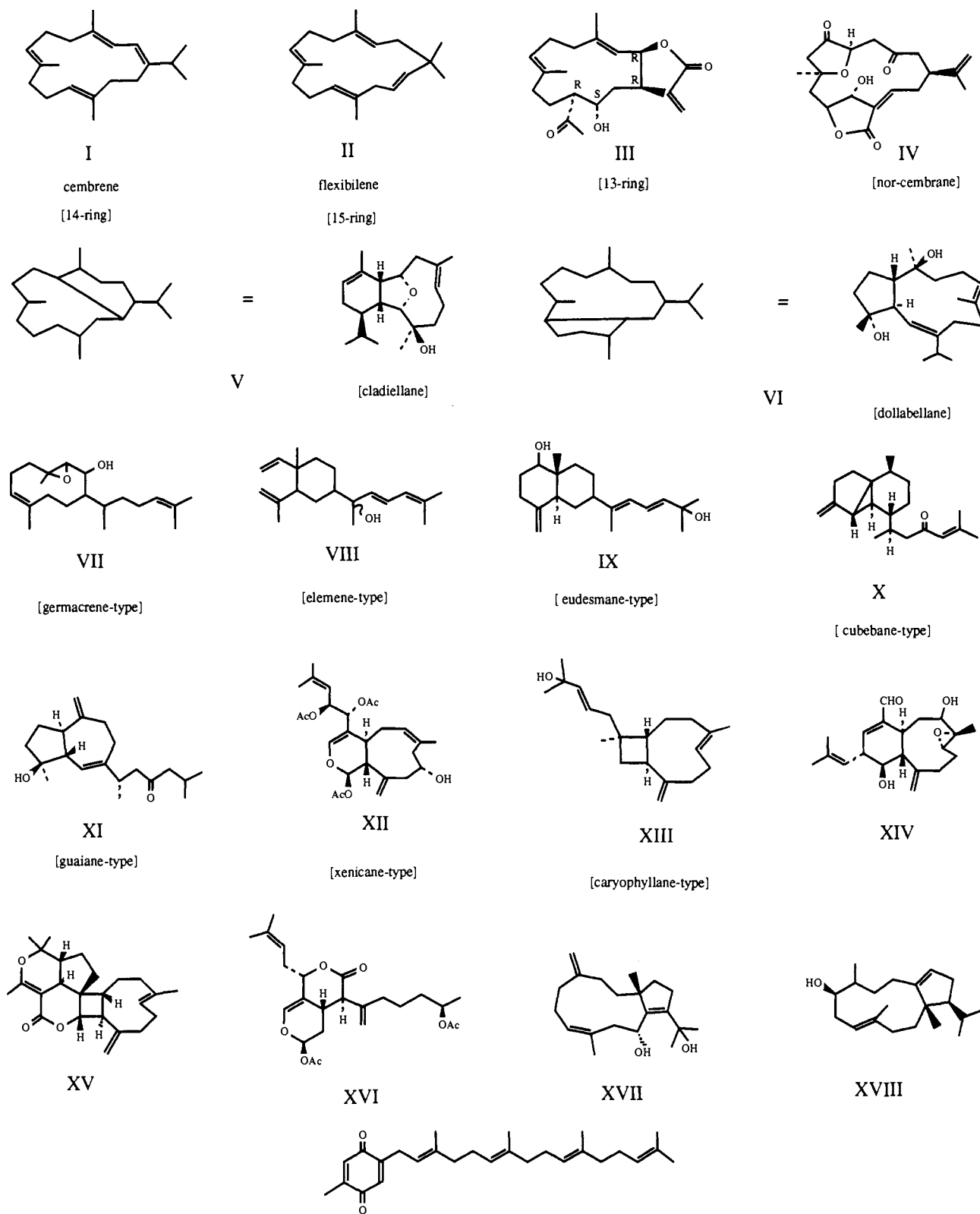
3. In the family Xeniidae

(a) *Efflatounaria* and *Cespitularia* spp.: They generally contain diterpenes and sesquiterpenes in the same colony. Cembranoid diterpenes (structure type III) occur only in these two genera within the *Xeniidae*, at about the 40% level.

(b) *Xenia* and *Anthelia* spp.: 90% of the diterpenes

TABLE II. Distribution of Diterpene and Sesquiterpene Types across the Alcyonacea (See Schemes I and II for the Structural Types)

FAMILY: ALCYONIIDAE	Diterpene Type (No. of examples)	Sesquiterpene Type (No. of examples)
Genus:		
Sinularia (15 species)	I II IV V *(33)(1) (4) (1)*	A B C D *(13)(1) (2) (1)*
Lobophytum (18 species)	I III VII VIII IX X *(32)(1) (3) (7) (3) (1)*	NONE
Sarcophyton (10 Species)	I + Dimeric *(33)* Diterpenes I	NONE
Cladiella (3 Species)	V *3*	NONE
Parerythropodium (2 Species)	NONE	E *(3)*

FAMILY: NEPHTHEIDAE

Genus:		
Nephthea (9 Species)	I XIII XIX *(7) (2) (3)*	F G H I J *(2) (1) (2) (1) (1)*
Lemnalia (7 Species)	NONE	D F K L M J N O *(2) (2) (6) (2) (1) (13) (2) (1)*
Litophyton (3 species)	I V *(3) (3)*	NONE
Capnella (1 species)	NONE	P Q *(1) (14)*
Paralemnalia (2 species)	NONE	K *(8)*

FAMILY: XENIIDAE

Genus:		
Xenia (12 species)	VII XI XII XIII *(2) (4) (41)(13)*	C J Q *(1) (3) (3)*
Heteroxenia (1 species)	NONE	J *(3)*
Anthelia (2 species)	XII XV *2 2*	NONE
Efflatounaria (5 species)	I XIVXVI *(2) (1) (4)*	F *(2)*
Cespitularia (5 species)	I XVII XVIII *(2) (1) (2)*	C F T *(4) (2) (1)*

FAMILY: ASTROSPICULARIIDAE

Genus:		
Astrospicularia (1 species)	XII *2*	NONE

SCHEME I. Diterpene Structural Types Exemplified by Specific Metabolites from the Alcyonacea (Roman Numerals Refer to Skeletal Type)

I

cembrene

[14-ring]

II

flexibilene

[15-ring]

III

[13-ring]

IV

[nor-cembrane]

V = [cladiellane]

VI = [dollabellane]

VII

[germacrene-type]

VIII

[elemene-type]

IX

[eudesmane-type]

X

[cubebane-type]

XI

[guaiane-type]

XII

[xenicane-type]

XIII

[caryophyllane-type]

XIV

XV

XVI

XVII

XVIII

XIX

from *Xenia* spp. and 100% from *Anthelia* spp. are based on the xenicane skeleton (types XV, XVI, and XVIII; see Scheme I).

4. Limitations on Reliability

While these generalizations may provide some support for classical taxonomic schemes, the points made

earlier by Tursch still hold true.[24] The reported presence of sesquiterpenes in corals lacking diterpenes is probably significant. The reported occurrence of diterpenes in corals presumed lacking sesquiterpenes may not be correct. Sesquiterpene hydrocarbons are volatile (i.e. lost on freeze drying unless care is taken[26]) and susceptible to oxidation once purified. Most soft coral

SCHEME II. Sesquiterpene Structural Types Exemplified by Specific Metabolites from the Alcyonacea (Capital Letters Refer to Skeletal Type)

A

B

C

D

E

Sesquiterpenes from the Alcyoniidae

F

G

H

I

J

K

L

M

N

O

P

Q

Sesquiterpenes from the Nephtheidae

R

S

T
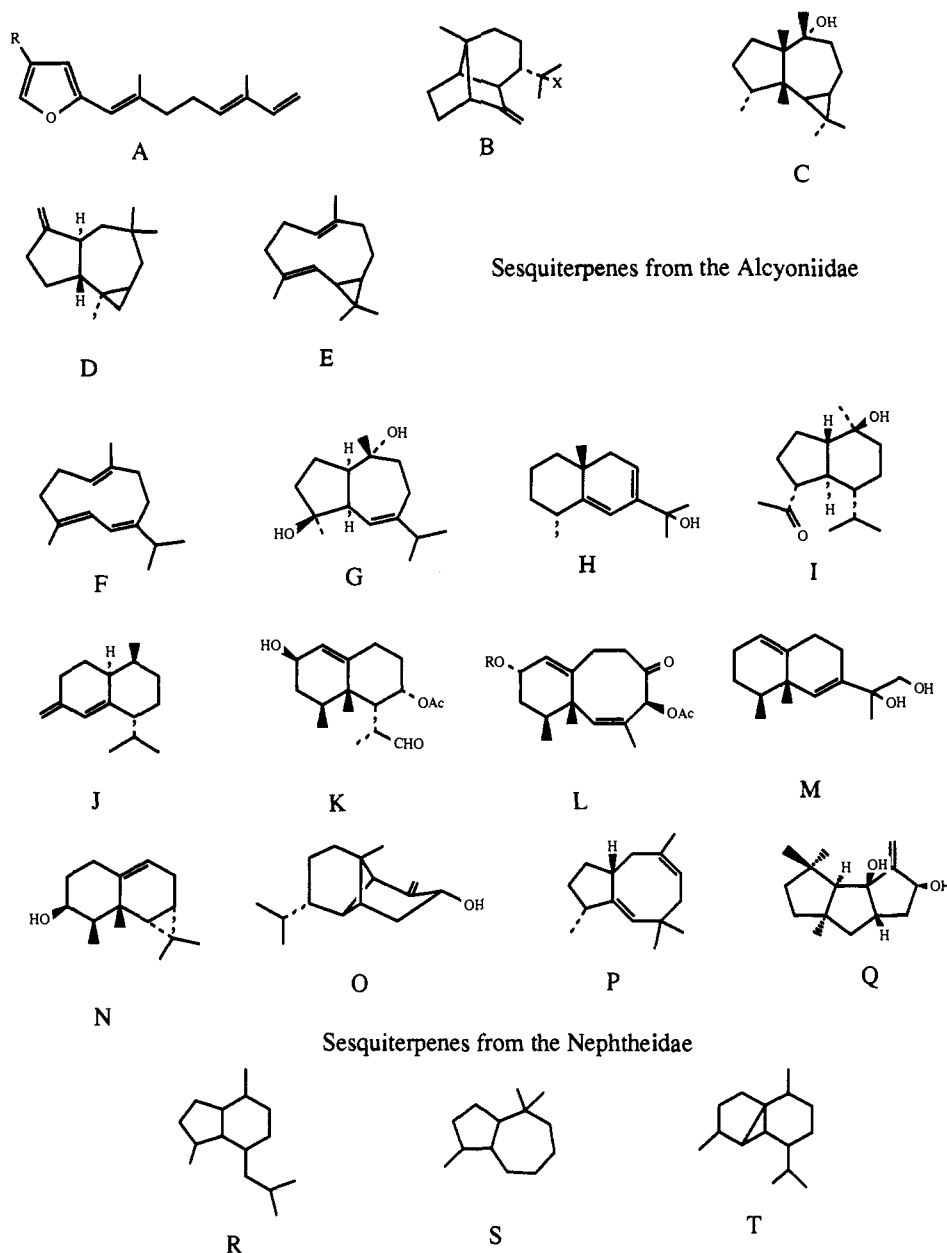
natural products chemists tend to ignore these nonpolar, relatively transient compounds when they accompany abundant and often crystalline diterpenes.

A second point is that the literature of the 1960s through the mid 1970s contains reports based on research using continuous wave or pulsed Fourier transform 80–100-MHz NMR spectrometers and often did not involve applications of HPLC. Many trace metabolites were not isolated, or if isolated, were not present in sufficient quantity (>50 mg) to permit complete structural elucidation with the available technology. Other reservations about the chemotaxonomic approach have been enunciated elsewhere.[33]

B. Biosynthetic Derivation of Octocoral Metabolites

As mentioned earlier, soft corals and gorgonians are often symbiotic associations of coral polyps with the

endo-symbiotic algae *Symbiodyniummicroadriaticum*.[6] In the early days of soft coral chemistry, cembranoid diterpenes appeared to be ubiquitous among octocorals. The other major source of cembranoid diterpenes was plants of the *Tobacco* species.[34] Presumably, reasoned Scheuer in 1973,[1] the symbiotic algae produce the terpenes.

In the last twenty years, the story has seesawed between the animal or plant origins of the octocorallian terpenoids. Several biochemical studies have implicated axenic cultures of zooxanthellae in the biosynthesis of precursors to,[35] and total biosynthesis of, the terpenoids found in octocorals.[36] On the other hand, studies using stable isotope ratio analyses of various metabolites concluded that the coral polyp was the active agent in the terpenoid biosynthesis.[37] The latter research group showed that axenic cultures of symbiotic algae produce different sterols than they do in the intact symbiosis.[38] In other words, there are two apparently conflicting sets

of results. The only definitive and unambiguous statement one can make is that some octocorals (e.g. *Pseudopterogorgia acerosa*), which do not contain symbiotic zooxanthellae, do contain significant quantities of highly functionalized diterpenes. These are the pseudopterolides (e.g. 1, Chart I), and it seems unlikely that they are of dietary origin,[39] although the possibility has not been ruled out by experimentation.

It thus seems that terpene biosynthesis is not dependent on the presence of zooxanthellae, although they may facilitate the process and permit larger quantities of a given metabolite to be synthesized. Preliminary studies of alcyonacean soft corals of the genus *Sinularia* suggest that mevalonolactone is incorporated selectively into the sesquiterpene portion of an arylated sesquiterpene 2,[40] and nonselectively into diterpenes derived from the genus *Sarcophyton*.[41] While radiolabeled sodium acetate was incorporated only into the butyrate (but not acetate) ester functions of diterpene 3 derived from *Alcyonium molle*, the same precursor was incorporated into the sesquiterpene skeleton of metabolites (e.g. 4) derived from *Cespitularia sp.*[42] Two points emerge from these studies: First, soft corals are amenable to biosynthetic study using radiolabeled precursors in the sea water, and second, expected results are not always obtained. More experiments need to be carried out before octocorallian biosynthesis is fully understood.

Recent reviews by Mary Garson and Leon Ciereszko[43] highlight general aspects of marine biosynthesis.

C. Chemical Ecology

Soft corals often contain significant quantities of secondary metabolites. As already mentioned *Plexaura homomalla* contains 1-5% of prostaglandin PGA$_2$ and derivatives.[12] Another gorgonian, *Pseudoplexaura porosa*, has been observed to have crystals of crassin acetate (5) in its tissue.[44] Certain alcyonaceans, including *Lemnalia humesi* contain 3-5% of the volatile sesquiterpene (6) in their tissue,[45] while *Sarcophyton* or *Lobophytum* specimens occasionally contain up to 10% of their dry weight as a single diterpene.[40]

Presumably such large quantities of secondary metabolites are produced at what must be considerable cost, because they play functional roles in the survival of octocorals. These may include defensive, competitive, reproductive, and possibly pheromonal roles. Some of these will be illustrated in what follows.

We have already reviewed the chemical ecology of alcyonarian corals.[33] Rather than rewrite that review for a chemical audience, I feel that it would be better to focus on two groups of alcyonarians, the genus *Plexaura* (Gorgonacea, Plexauriidae), represented by *Plexaura homomalla* and selected other species and related genera, and the genus *Sinularia* (Alcyonacea, Alcyoniidae), represented by *Sinularia flexibilis* and other species. Each has generated a range of chemical reports and each has been studied because of the possible roles of its chemistry in ecology.

Before dealing with the chemical ecology of the Alcyonacea which is to a large extent based on work carried out in my group over the last 10 years, I will look first at the Gorgonacea, of which *Plexaura homomalla* is probably the most important, although certainly not the most typical species.

III. Chemical Ecology of the Gorgonacea

A. General Background

The chemistry of the Gorgonacea has been well documented, following the report by Ciereszko et al.[11] in 1960 which indicated the presence of biologically active diterpenes crassin acetate (5) from *Pseudoplexaura crassa* and eunicin (7) from *Eunicea mammosa*. These and other terpenes isolated, but not at that time structurally characterized, appeared to account for the majority of the biological activity of gorgonians previously noted by the Burkholders.[47] Over the next 20 years, many new compounds were isolated from the Gorgonacea, mostly falling into the sesquiterpene or diterpene classes.[24] Although the chemical literature of the group has continued to expand,[20] the isolates from northern hemisphere gorgonians are largely terpenoid in derivation. Indeed as noted earlier, Gerhart[25] was able to establish a chemotaxonomic basis for the Gorgonacea, using terpenoid characters. The major departure from the relatively predictable chemical profile of the Gorgonacea was *Plexaura homomalla*,[12] and the Indo-Pacific species *Isis hippuris*[48] which contain interesting sterols and lipid derived compounds. Indeed the discovery of prostaglandins in *P. homomalla*[12] was completely unexpected on the basis of the literature, and *P. homomalla* is very atypical of the northern hemisphere Gorgonaceans.

Research into the chemical ecology of the Gorgonacea that has been carried out has related largely to the effect of cembranolide diterpenes derived from gorgonians on ciliate or flagellate plankta, evidence for food chain linkages based on sterol distribution, and the chemical ecology of *Plexaura homomalla*, especially in relation to its defense based on the presence of derivatives of the prostaglandin PGA$_2$.

A major review of the chemistry and ecology of *Plexaura homomalla* appeared in 1974[13] as a result of a symposium held to assess the suitability of *P. homomalla* as a mariculture resource to supply prostaglandins for the pharmaceutical industry.

B. Ecological Experiments

1. Effects of Cembranolide Diterpenes on Marine Ciliates and Flagellates

Over a number of years, Leon Ciereszko[49] and his various biological collaborators have pursued the line that cembranolide diterpenes, often present at 1-2% of the dry weight of gorgonians, may be used in defense, interference competition, food capture, and even possibly the acquisition and selection of symbiotic zooxanthellae. These postulates are based on the following experimental studies.

In 1970, Ciereszko[50] showed that the marine ciliate *Tetrahymena pyriformis* suffered growth and motility depression in the presence of crassin acetate (5). This outcome supported the hypothesis that the diterpene decreased the viability of ciliated larvae of organisms which compete for available space with gorgonians. Somewhat later, it was shown that eunicin (7) caused loss of velar cilia (and eventual death) in the larvae of the nudibranch *Phestilla sibogae* Bergh.[51] It is possible that the loss of the velar cilia may serve to immobilize
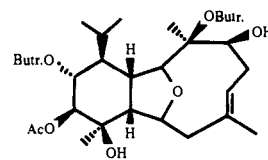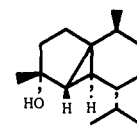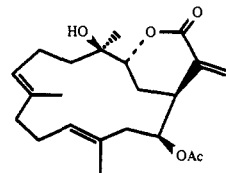
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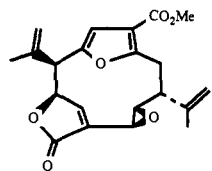
1
pseudopterolide
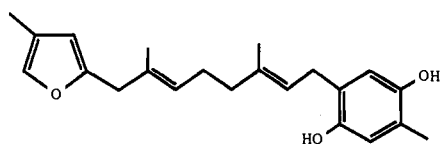
2
furanoquinol
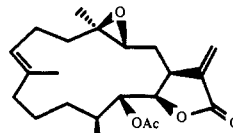
3

4

5
crassin acetate
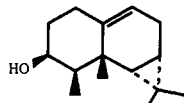
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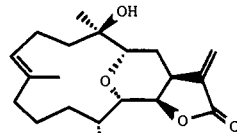
7
eunicin

8
eupalmerin acetate
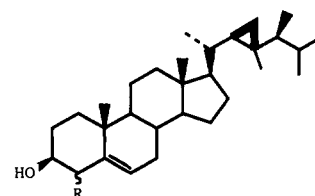
9

R=H: gorgosterol
R=Me: 4-methylgorgosterol
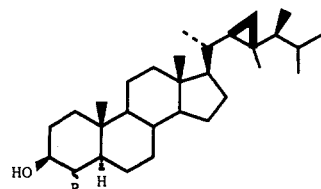
10

R=H: gorgostanol
R=Me: 4-methylgorgostanol
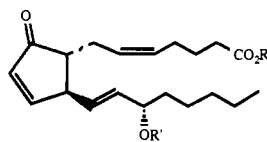
15R-PGA$_2$ derivatives

11 R=R'=H: 15R-PGA$_2$

12 R=R'=Ac: acetoxy methyl ester

13 R=Me,R'=H

14 R=H,R'=Ac
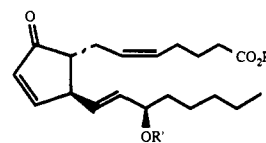
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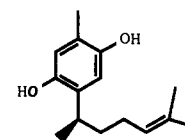
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curcuhydroquinone
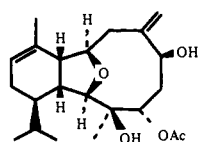
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alcyonin
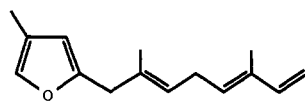
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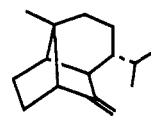
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sinularene
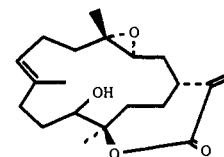
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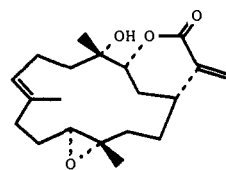
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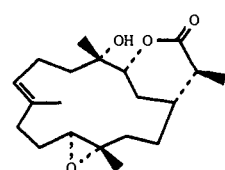
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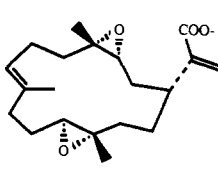
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bis-epoxide precuser
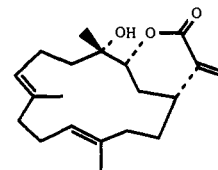
24
Uchio's toxin

25

26
pukalide

27
epoxypukalide

28
lophotoxin

the larvae—inducing them to settle on the coral. Another possible explanation is that this is merely a chemical competitive strategy which prevents the settlement of ciliated planktonic larvae in general, near the gorgonians.

Further experiments were carried out to see if the loss of cilia was a general occurrence or merely a species-specific response by *Phestilla sibogae* larvae.[51] A series of experiments were carried out on the rotifer *Brachionus plicatilis* Muller and the 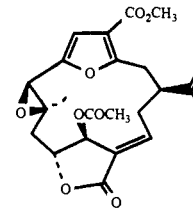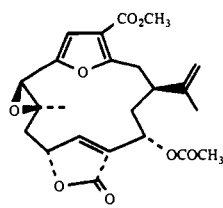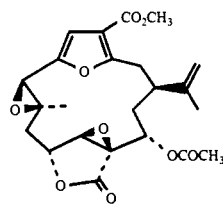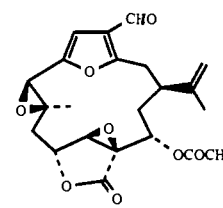amphipod *Parhyale hawaiiensis* Dana.[52] The cembranolides eunicin (7), crassin acetate (5), and eupalmerin acetate (8) each killed the rotifers and copepods at 10 ppm or less. In this case, there was no evidence of deciliation: They simply suffered loss of motility and eventual death, suggesting that the chemicals may play defensive and competitive roles in the gorgonians, reducing the fitness of competitors and would-be predators.

The most recent studies in this arena relate to the effect of cembranolides especially eunicin (7) on flagellate motility.[53] In almost all of the 28 flagellate phytoplankton cultures from a number of the algal families, eunicin at 5 ppm caused loss of motility. Crassin acetate (5) and eupalmerin acetate (8) were generally less effective than eunicin (7). Of particular interest was the effect of eunicin (7) on three cultures of *Symbiodinium* derived from different invertebrate hosts. The responses varied considerably from immobilization after 12–40 min to only modest effects on motility after 14 h. It was suggested that in some cases, the cembranolides may be used to immobilize phytoplankton for food. For a species (or type) of zooxanthellae (especially *Symbiodinium microadriaticum* which is involved in symbiosis with a specific coral species), it may be immobilized to aid in the assimilation and incorporation of the zooxanthellae into the coral's symbiotic system. The other role of the relatively toxic cembranolides is in chemical competition and defense.[53]

While many of the proposed ecological roles of the cembranolides are speculative, the speculation is based on carefully designed and executed laboratory experiments which provide circumstantial evidence to support the proposed ecological roles of the secondary metabolites. The work of Ciereszko and associates involves collaborations between chemists and biologists—structural, behavioral, physiological, and ecological factors have been given due consideration in the experimental design and in the discussions and implications of the results obtained in the experiments.

2. Food-Chain Linkages Based on Chemical Markers

A second area of gorgonian ecology with which Leon Ciereszko and collaborators have been involved relates to the possible use of the unusual C_{30} sterols gorgosterol, gorgostanol, and their methyl derivatives as diagnostic markers in the identification of food sources. It is likely that the gorgosterols are derived from the symbiotic zooxanthellae of many invertebrate hosts.[6] The findings of the study[54] may be summarized as follows:

(1) The flamingo tongue cowrie *Cyphoma gibbosum* feeds on the gorgonian octocoral *Briareum asbestinum*; the sterol pattern of *C. gibbosum* and of its fecal pellets was qualitatively the same as that of *B. asbestinum*. The large amounts of gorgosterol (9) in the feces

probably accounts for its presence in marine sediments.[55]

(2) The alcyonacean *Xenia* sp. also contains gorgosterol (9) and gorgostanol (10) which were detected in the tissues of its selective predator—the crab *Caphyra laevis*.[56]

(3) The crab *Domecia acanthophora* has a commensal relationship with the hard coral *Acropora palmata*. Analysis of the crab (and coral) revealed the presence of gorgosterol (9) and gorgostanol (10). This was also found for the crab *Mithraculus sculptus* and the coral *Porites porites* var. *furcata* which existed in a commensal relationship.[54]

(4) *Hermodice carunculata*, the coral eating fire worm, feeds on the hard coral *Porites porites*, gorgonians, zoanthids etc. The sterol pattern of *H. carunculata* again contains the C_{30} sterols.[54]

The study thus revealed that analyses of fecal pellets or tissues of predators could confirm the nature of alleged predator–prey associations.[54]

IV. The Chemical Ecology of Plexaura homomalla

A. Chemical Aspects

The chemistry and chemical ecology of the Caribbean gorgonian *Plexaura homomalla* is by no means simple. As mentioned earlier, the 1969 report of Weinheimer and Spraggins indicated the presence of (15R)-PGA$_2$ (11) and its methyl ester acetate (12).[12] These were present in 0.2 and 1.3% of the air-dried weight respectively. Subsequent reports suggest that if the colonies are frozen (liquid N$_2$) immediately on collection, then only the acetate of (15R)-PGA$_2$ methyl ester (12) is detectable.[57] Hydrolysis (enzymic and/or chemical) can thus result from inappropriate collection and handling and lead to four compounds in all: 11, 12, the hydroxy methyl ester (13), and the acetoxy acid (14). It is likely that the same range of metabolites would be generated when the gorgonians' tissue is eaten both at the site of the wound by enzymic action and within the gut of the fish, due to the pH and/or the presence of hydrolytic enzymes.[58]

Other collections of *Plexaura homomalla* afforded derivatives of (15S)-PGA$_2$ (15).[59] Again careful collections afforded only the acetoxy methyl ester derivatives of (15S)-PGA$_2$, while other less careful collections contained the free acids and monoesterified derivatives analogous to 11–14. The (15S)-PGA$_2$ derivatives were the mammalian compounds, and so, in terms of normal (mammalian-based) bioassays, they were more active than the (15R)-derivatives. This is of little relevance to the coral, except that the (15S)-PGA$_2$-containing corals are more likely to be harvested for their chemical constituents by the pharmaceutical industry.[13]

A careful taxonomically based study by Ciereszko et al.[60] discussed the existence of two varieties of *Plexaura homomalla* Esper: forma *homomalla* and forma *kukenthali* Moser. The latter, which cooccurs with the usual *P. homomalla*, contained only (15S)-PGA$_2$ derivatives. It seems possible that there are two types (species?) of *P homomalla*, one of which produces (15R)-PGA$_2$ derivatives, while the other produces (15S)-PGA$_2$ derivatives. Collections which contain

mixtures of (15R)- and (15S)-PGA$_2$ derivatives may be mixed collections.[60]

B. Ecological Aspects: Defense

1. Feeding Deterrence

Several studies of *Plexaura homomalla* and its metabolites are relevant to its chemical ecology. *P. homomalla* is one of the most important constituents of the attached benthic faunal biomass in the Caribbean reaching 50% levels in some areas.[13] It is exposed, obvious, and a rich source of lipids and other essential nutrients. It does not appear to suffer heavy predation from generalist predators. Studies by Gerhart[58] revealed that the aqueous extracts of *P. homomalla* were toxic to goldfish and that the total lipid extract when added to food pellets caused rejection of the pellets by killifish. Any fish that did ingest the pellets rejected subsequent offers of treated pellets, although they happily consumed the control pellets and other food provided.[58] Experiments in which pure (15R)-PGA$_2$ was added to pellets again resulted in high rejections and eventual total rejection by killifish. Force feeding of killifish with PGA$_2$-treated pellets resulted in 80% regurgitation, although controls showed no ill effects. Field trials with (15S)-PGA$_2$ or (15R)-PGA$_2$ resulted in vomiting by wrasses within 2–5 min. All untreated pellets were consumed. Rejection of all treated pellets occurred in subsequent offering trials.[58] In these studies, crude extracts and pure PGA$_2$ (15R or 15S) acted as feeding deterrents and, if ingested, generally produced vomiting. This was a particularly detailed study, executed over a long period of time.[58] It contains significant and useful results.

A more recent study by Pawlik and Fenical confirmed the above findings[61] but questioned the conclusion that PGA$_2$ was an agent of chemical defense, because live gorgonians do not contain free PGA$_2$ acids. The fully esterified PGA$_2$ derivatives (e.g. 12) present in the living corals did not show feeding deterrence in the field as assessed by Pawlik and Fenical,[61] although the partially hydrolyzed derivatives 13 and 14 were quite deterrent. The criticism has some validity, but in some ways it appears to strengthen the conclusions of Gerhart's study.[58] What the study showed was that totally protected PGA$_2$ compounds do not cause vomiting or feeding aversion. One criticism which may be made of the later study was that the authors failed to observe the fish eating the test strips and did not observe the fish which had consumed the food. It is therefore not known whether the fish later regurgitated the food after ingestion. Because they did not observe the effects of the ingestion and perhaps possible regurgitation,[61] it does not seem that the later study adds greatly to the information provided by the earlier study.[58]

On the basis of Gerhart's study,[58] it seems probable that the ingestion of significant amounts of *Plexaura homomalla* tissue by a generalist predator will result in partial or total hydrolysis[61] leading to subsequent vomiting and aversion to eating *P. homomalla* tissue. Fish learn very quickly, and contrary to the suggestion of Pawlik and Fenical,[61] I believe that such a delayed response would still possess a feeding deterrence value for *P. homomalla*, probably almost as effective as an initial bad taste. We might refer to it as a "post bad

restaurant syndrome". It is also possible that limited opportunistic grazing may play a role in the life cycle of *P. homomalla*. Some gorgonians and sea pens (Pennatulacea) use a form of fragmentation-based asexual reproduction. After injury, a segment of a branch may budd off and fall to the ground where it establishes a holdfast and becomes a new colony. This is especially likely at the actively growing polyp-bearing branchlets which are most favored by fish. Thus, limited fish grazing by naive fish may be an important aspect of the reproductive strategy of *P. homomalla*. Excessive grazing is controlled by the delayed emetic effects of the PGA$_2$ components.

A recent paper by Gerhart uses the term "emesis" for this learned aversion phenomenon.[62]

On the basis of Gerhart's[58] and Pawlik and Fenical's[61] studies, it seems likely that the prostaglandins of *Plexaura homomalla* do provide effective defense against predation from generalist or opportunistic feeders. Subsequent alimentary disruption usually mitigates against a return to an offending restaurante by *Homo sapiens*, and when it comes to gustatory matters, fish have very high I.Q.'s!

2. Reproduction

Ciereszko et al.[60] also analyzed the lipid content of the eggs of *Plexaura homomalla*, which they excised from colonies in December. The eggs contained no prostaglandins. This may not be as significant a result as it seems. Eggs of *P. homomalla* are generally mature in late summer (July, August). On this basis, the eggs studied were probably quite immature, and work on alcyonacean eggs reveals that egg-specific compounds (including in one case, the acetoxy methyl ester of PGA$_2$) are only present in the eggs the month before release.[63]

3. Coevolved Predator—Cyphoma gibbosum

A second class of predator uses *Plexaura homomalla* as a common food source. *Cyphoma gibbosum*, the flamingo tongue snail, is frequently found eating *P. homomalla* with no apparent ill effects. Indeed, *C. gibbosum* feeds on colonies of *P. homomalla* in groups or clusters, usually consuming tissue from the sterile (nonpolyp-bearing) stalk region. This behavior, and any chemical basis leading to it, have also been the subject of a number of studies by Don Gerhart.[64] In a detailed study, he showed that there was no correlation between the chemical composition (by TLC) of gorgonian colonies occupied by clusters of *C. gibbosum* when compared with those unoccupied by the mollusks.[64] There was a significantly higher than expected observation of mollusks clustered on *P. homomalla* colonies, while singly occupied colonies were significantly below expected levels. An interesting result was that previously grazed colonies were only occupied for 1–2 days on average, while previously unoccupied colonies were grazed for more than 9 days.[64] This may be due to the fact that the quantity of tissue was limited after the previous occupancy, but this did not appear to be the case on inspection. It was however consistent with the fact that at the site of tissue damage, enzymic hydrolysis releases partially or totally hydrolyzed PGA$_2$ derivatives[59] which have been shown to be feeding deterrents for a number of fish[61] and to produce an emetic effect

when force fed to fish.[58] Perhaps the gregarious behavior of *C. gibbosum*, which is facilitated by mucous trail following behavior, is based on the rapid ingestion of tissue prior to the extensive enzymic hydrolysis which follows tissue destruction. With the subsequent progressive taste deterioration of the colony, the group of mollusks moves on to an as yet unassailed colony, following a mucous trail left by the leader. Apparently the emetic response, so obvious in the case of fish, is not relevant to these mollusks once the tissue reaches the digestive gland, a specialized organ involved in processing often toxic metabolites in the diet of selective predatory mollusks. (see ref 65 for example.) Predation by *C. gibbosum* is not totally benign as revealed in a recent study by Gerhart.[66]

Analysis of *Cyphoma gibbosum* tissues and fecal pellets by Ciereszko and associates,[67] revealed that PGA_2 and derivatives are indeed present in the snail. Its deposition products, the fecal pellets, did not appear to contain prostaglandins. The reported conversion of PGA_2 to PGB_2 derivatives in the snail may be correct but may also be the result of acid treatment during extraction and workup.[67] It seems likely the *C. gibbosum* does indeed store prostaglandin derivatives derived from ingestion of *Plexaura homomalla* and that these partially or totally hydrolyzed metabolites confer on *Cyphoma* a distastefulness which deters predators. *C. gibbosum* is brightly colored, feeds in groups without seeking refuge in cryptic areas, and seems to flaunt its distastefulness, presumably acquired from the ingestion of large amounts of *P. homomalla*.

Further explanations for the clumping of *Cyphoma gibbosum* on the basal regions of *Plexaura homomalla* comes from two studies. The first by Gerhart[68] is a study which shows that physical factors (waves, surge, current, etc.) favor settlement of *C. gibbosum* on the lower, more stable, nonpolyp-bearing regions of the gorgonian colonies. The second is an excellent example of a study in chemical ecology carried out by the Harvell and Fenical.[69] This study dealt with the distribution of chemical and physical defenses in *Pseudopterogorgia* species, a genus with similar physical attributes to *Plexaura homomalla*. In this elegant study they showed that the ichthyotoxins pseudopterolide (1) in *Pseudopterogorgia acerosa* and curcuhydroquinone (16) in *P. rigida* were located almost entirely on the actively growing polyp-bearing branchlets, while the sterile (nonpolyp-bearing) regions of the colonies, usually nearer the holdfast, were richly endowed with sharp, protective spicules (limestone microparticles). There was an inverse correlation between toxicity and spiculation.[69] Fish, opportunistic grazers, usually pick the chemically protected, polyp-bearing branchlets of soft coral colonies and are deterred. Specialist predators ("coevolved predators") such as *Cyphoma gibbosum* which does not have problems ingesting and processing spicules, concentrates on the lipid rich, but spicule-packed, sterile tissue. This may become more distasteful due to hydrolysis of the protected PGA_2 derivatives and hence somewhat deterrent to *C. gibbosum* after extensive grazing, or there may not be sufficient nutritional value left for a number of mollusks to satisfy their needs. The "herd" or "flock" of mollusks moves on to the next colony.

C. Ecological Aspects: Competition

1. Antifouling and Allelopathy

A recent chapter in the chemical ecology of *Plexaura homomalla* was written in relation to the possible involvement of prostaglandin derivatives in the prevention of fouling of colonies and in the interspecific competition between attached organisms by allelopathy. A number of the cembranolides have been implicated as antifouling[49] or anticompetitor agents[53] due to their slow release from the gorgonian colonies. Gerhart[70] used osmotic minipumps to deliver either (15R)- or (15S)-PGA_2 (or seawater as control) to gorgonian skeletons freed of tissue. Analysis of fouling of the skeletons revealed that the treatments had no significant effect on fouling of the gorgonian skeletons. In a second series of experiments, Gerhart[70] used reversed-phase Sep-Paks to see if significant quantities of prostaglandins were released into seawater around the gorgonian *P. homomalla*. The study concluded that no significant quantities of prostaglandins were released into seawater around uninjured colonies of the gorgonian *P. homomalla*. Gerhart thus concluded that the primary role of the PGA_2 derivatives was as defensive agents against generalist predators. They do not make significant contributions to the prevention of fouling of the colonies nor are they involved in interspecific competition via allelopathy.[70]

The final chapter in this story illustrates the concept of a "kairomone" as enunciated by Whittaker and Feeny in 1971:[71] "a compound released by one species which attracts would-be predators". It appears from studies carried out by Wahle,[72] that the hydrozoan corals *Millepora alcicornis* and *M. complanata* are able to detect chemical signals from nearby *Plexaura homomalla* colonies. They redirect their growth toward the colonies, and eventually overgrow and kill *P. homomalla*. Too much chemistry may be a health hazard!

2. Concluding Remarks

Gerhart's contributions to the literature in the area of gorgonian systematics and ecology, involving secondary metabolites as the characters, vectors or agents, have been most significant. The major limitation of this work is that Gerhart is a biologist/ecologist trying to do the tasks of chemist and ecologist. While he has recorded significant achievements in the field, I believe that this work could be even more significant, if he were to work in close collaboration with chemists. While TLC may be adequate for a number of purposes, it is not a good means for quantification (cf. NMR or HPLC) and is not unambiguous for the identification of metabolites especially prostaglandins in the presence of other fats, unless it is coupled with other techniques (especially NMR, IR, UV, etc.) for substantiation of the results. Compounds often change with time and their purity should be confirmed just before an ecological experiment is performed (at least by NMR). Gerhart's work could be significantly better if the studies were run in conjunction with chemists. Hopefully this will occur in the future.[73]

V. Chemical Ecology of the Alcyonacea

For the last 20 years, chemists at James Cook University in Townsville have been engaged in studies of the
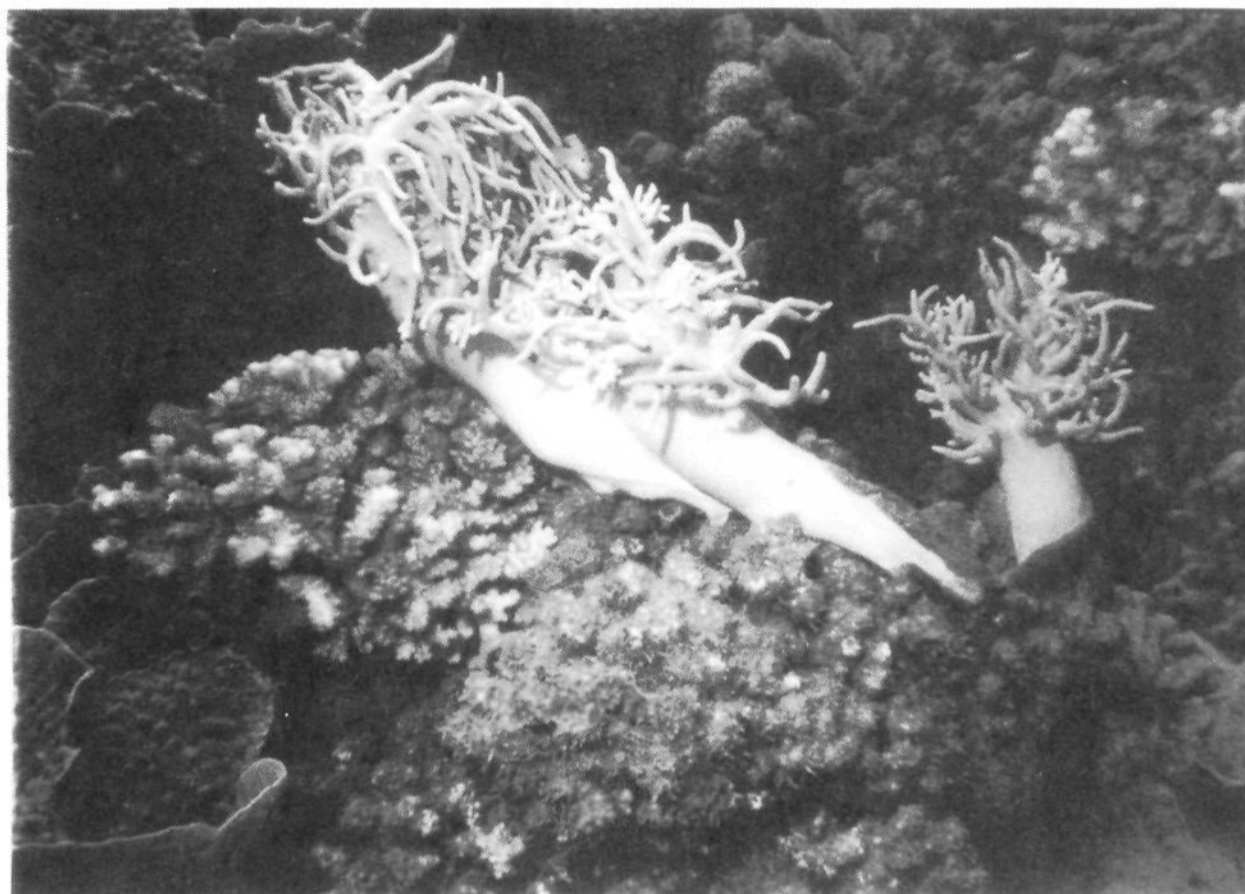
Figure 3. Colonies of the soft coral *Sinularia flexibilis* showing its mode of movement overgrowth across the surface of living hard corals. Note also colony fission is occurring in the larger colony, eventually giving rise to two individual clonal colonies.

chemical constituents of alcyonacean soft corals. These studies have resulted in almost 60 papers in the "Studies of Australian Soft Corals" series, most of which have appeared in the *Australian Journal of Chemistry*. In addition to these purely structural investigations, we became interested in the ecological reasons for the structural chemical diversity. This change of focus was inspired by some of the early work by Tursch and Ciereszko and by contact with ecologists like Jerry Bakus. It was also mandated upon us by virtue of having a well-equipped chemical laboratory with state-of-the-art analytical equipment located beside the Great Barrier Reef, Australia.

A. General Background

1. Sinularia flexibilis and Other Sinularian Corals

The taxonomic identification of the genus *Sinularia* is relatively straightforward. The basal region of virtually all sinularian corals is densely packed with spicules which are usually needles or spindles, between 1–5 mm in length. The spicules are easily seen underwater, simply by cutting the base with a knife. This is the only genus with this characteristic. Within the genus, there are almost 90 species, and thus species identification is not trivial, although there is a very good taxonomic key available.[74]

Within the genus, chemistry is quite varied and spans the range from sesquiterpenes to diterpenes as shown in Table II. Several of the species might have been loosely ascribed to *Sinularia flexibilis* by inexpert taxonomists. The recent unexpected report of a cladiellane-based diterpene (**17**) from *S. flexibilis* may be an example of this,[75] although a taxonomic specimen recently provided by Kusumi was *S. flexibilis*.

Only two species of *Sinularia* have yielded sesquiterpenes in addition to those included in Kashman's chemotaxonomic study.[27] The first is *Sinularia capillosa* (cf. *gonatodes*) which contained a series of fu-

ranosesquiterpenes[46] (e.g. **18**). It is possible that these might be precursors of the furanoquinol (**2**),[76] whose biosynthesis was reported in a related paper.[40] The other sinularian coral to yield sesquiterpenes was *S. mayi* which afforded the unusual tricyclic sesquiterpene **19**.[77]

Sinularia flexibilis is among the most beautiful soft corals in the Indo-Pacific region (Figure 3). It is cosmopolitan in its distribution, and its highly spicularized sterile stalk is in sharp contrast with the polyp-bearing branches and branchlets, which are almost devoid of spicules. The chemical attributes of the colony more than compensate for any lack of physical protection.

Figure 4 shows the range of diterpenoids reported from *Sinularia flexibilis*. Although *S. flexibilis* has been reported to contain only diterpenes, it is quite likely that colonies do contain amounts of sesquiterpene hydrocarbons. These are rarely identified because of their volatility (lost on freeze drying) and instability and because of the predominance of crystalline diterpenes in the coral extracts.

B. Ecological Aspects: Defense

1. General

Our earliest endeavors in this new area involved the use of *Gambusia affinis*, the common freshwater mosquito fish, as an indicator of the biological activity of extracts and pure compounds. We used them to measure ichthyotoxicity and later the feeding deterrence properties of aqueous extracts of soft corals.

It was reasoned that predation on a soft coral would release a range of organic molecules into the surrounding sea water and that the properties of this aqueous suspension could influence subsequent predation, either due to toxicity or taste. The nature of the aqueous suspension derived from maceration of soft corals might thus contain toxins and also gustatory and/or olfactory signals as warnings against predation.
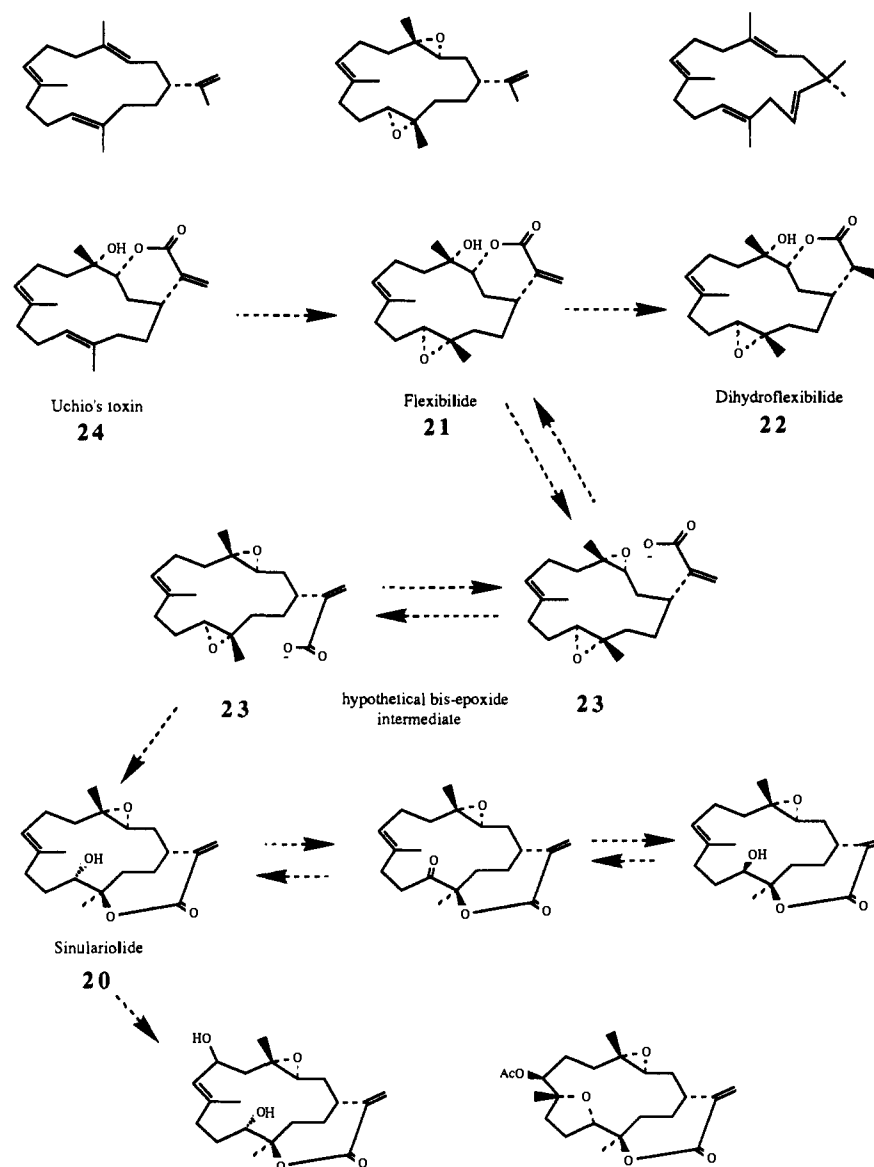
Figure 4. Known metabolites isolated from *Sinularia flexibilis* showing possible enzymatic interconversions. Numbers are only ascribed to compounds discussed in the text.

Using techniques devised by Yamanouchi[78] and developed by Bakus,[79] we macerated a fixed amount of frozen coral in distilled water and tested the supernatant liquid after centrifugation, by adding it to small fresh water aquaria containing the mosquito fish. Behavior changes were noted in the fish and mortality counts taken at regular intervals.[80] In this way, the aqueous extracts of more than 150 soft corals collected over a span of 1000 km of the Great Barrier Reef were assessed. As noted elsewhere,[33] about 60% of the soft corals (large, common, and exposed rather than cryptic) were toxic to the mosquito fish, *Gambusia affinis*. Clearly many of the soft corals contained very bioactive and toxic molecules. The corollary of this result however, is that about 40% of the soft coral extracts were nontoxic. If ichthyotoxicity was a protection against generalist predators, what protected the rest of the nontoxic corals?

A significant number of soft corals were also shown to be distasteful to the test fish. This time, the aqueous macerate was absorbed into the regular fish food used by *Gambusia affinis* and freeze dried before being of-

fered to the fish. Treated and untreated food were assessed for acceptance by the fish. Almost 90% of the extracts were distasteful at the highest concentration. Acceptance reached about 50% when the concentration was only 25% of the maximum level tested. Perhaps surprisingly, ichthyotoxicity and feeding deterrence were not correlated. That is, as many of the very toxic extracts were distasteful as were nontoxic ones.[81] It could then be assumed that a further 50% of the nontoxic extracts would have significant feeding deterrence.

What other form of protection was possible? Most soft corals possess limestone spicules, and some of these have significant deterrent value. They protrude in such a way as to protect the polyps (Figure 2a, *Nephthea* sp.) or are so densely packed in the tissue (Figure 2b, *Sinularia* sp.) as to provide a significant disincentive to predators. Indeed, a recent study of gorgonian extracts has shown that the presence of spicules in test food deters consumption. Indeed, it appears that the deterrent attributes of the organic extracts are significantly enhanced by the presence of spicules in the test food.[82]

2. Sinularia flexibilis and Ichthyotoxicity Studies

The ichthyotoxicity of the aqueous extracts of 160 soft corals from all families was 60%.[33] The value for the genus Sinularia was 63% i.e. the genus Sinularia was not significantly more toxic than all other soft corals considered together. Sinularia species however were disproprotionately represented in the most toxic group of extracts. Thus 50% of the most toxic group (all fish dead within 45 min) were from the genus Sinularia.[80] Aside from being chemically rich and varied, many of the metabolites are clearly ichthyotoxic.

Sinularia flexibilis contains varying amounts of the three major metabolites sinulariolide[83] (20), flexibilide[84] (sinularene)[85] (21), and dihydroflexibilide[84,85] (22). The two parent diterpenes sinulariolide (20) and flexibilide (21) may be thought of as deriving from a common presursor—the bis-epoxide 23. The published X-ray data for sinulariolide[86] (20) reports the absolute configuration of the metabolite, while that of the flexibilide (21) describes its relative configuration.[84] If the bis-epoxide 23 is set up with the correct absolute configuration to afford sinulariolide on lactonization and epoxide opening, it can also afford the enantiomer of the structure drawn for flexibilide[84] by the alternative lactonization and epoxide opening (Figure 4).

The ichthyotoxicity of Sinularia flexibilis may be assumed to be derived from these major metabolites. However, Tursch reports that sinulariolide (20) was not ichthyotoxic,[24] and recent very careful work by Uchio[87] has shown the flexibilide (21) is also not toxic. The toxin in S. flexibilis 24 is flexibilide lacking the 11,12-epoxide group. It is more than 100 times more toxic than flexibilide (21) and was only detected and identified by bioassay directed fractionation. Further careful analyses of S. flexibilis colonies from various Indo-Pacific reefs have afforded the range of metabolites listed in Figure 4.

In general, the ichthyotoxicity of soft corals can be largely attributed to the presence of cembranoid diterpenes in the corals, which are often ichthyotoxic to Gambusia affinis at 5–20 ppm. Soft corals lacking physical defense thus appear to be protected from predation by the presence of diterpene toxins in their tissue, and this is most obvious among soft fleshy members of the genus Sinularia. It is worth considering what roles might be played by sinulariolide (20) and flexibilide (21), since they are major constituents in most colonies of S. flexibilis and yet they do not contribute to ichthyotoxicity.

3. Sinularia flexibilis and Feeding Deterrence

Sinularia flexibilis as indicated earlier possesses an ichthyotoxic aqueous extract which killed all Gambusia affinis in less than 45 min.[80] This extract also contains components which are distasteful to fish. While the toxicity has been shown to be associated with a cembranolide diterpene 24 related to flexibilide (21), both the aqueous and DCM-soluble subfractions of the aqueous macerate of S. flexibilis are ichthyotoxic. Feeding deterrence, however, is more strongly associated with the aqueous (rather than the DCM) soluble fraction.[88] This suggests that ichthyotoxicity and feeding deterrence are not necessarily mediated by the same suite of compounds. Considering that there was no association between ichthyotoxicity and feeding

deterrence for a range of soft corals, this suggestion seems quite reasonable. Of the eight innocuous sinularian corals assessed for feeding deterrence in the earlier study,[80] four showed significant feeding deterrence, reinforcing the notion of a complementarity of defenses between ichthyotoxicity and feeding deterrence.

A recent study by Wylie and Paul[89] showed that, for three species of Sinularia in Guam, significant feeding deterrence, as assessed by local reef fish, was associated with the lipid-soluble extracts of the corals at or below their natural concentrations. The deterrent molecule from S. maxima was identified as the cembranolide diterpene 25, previously isolated from S. polydactyla in Australia.[90]

4. Physical vs Chemical Defense—A Role for Inorganic Chemistry in the Genus Sinularia

As already noted, about 60% of sinularian colonies possess ichthyotoxic properties. About 50% of the nontoxic corals possess feeding-deterrent properties. Sinularian soft corals range from the fragile, but toxic beauty of Sinularia flexibilis (Figures 3 and 5) to the obdurate hardness of the encrusting spicule packed S. dura (Figure 2b). This range of morphology enabled us to code colonies for physical protection derived from the presence of spicules in the colony. The coding tried to take account of the difficulty of consuming the polyps or fleshy coenenchyme from the perspective of predatory fish. When this was done for 28 sinularian corals, it was found that there was an inverse correlation between physical defense (especially the presence of a spicule-packed coenenchyme) and toxicity. A similar inverse correlation was obtained for the family Nephtheidae, where the physical protection related to the armament of the polyps (see Figure 2a).[91]

In summary, the genus Sinularia uses chemical defenses in the form of terpenoid ichthyotoxins and a range of feeding deterrent molecules when the colony form is soft and fleshy and relies on inorganic chemistry—a spicule-packed coenenchyme when the colony is low and encrusting and lacking chemical defense.

C. Ecological Aspects: Competition

1. General

While it is important for soft corals to deter predation using a range of organic and inorganic chemical strategies, it is also important that they be able to compete for space on the solid surfaces afforded by a coral reef. Competition for space is probably one of the most important factors which determines the distribution of species on a coral reef.[92] The term "interference competition" implies that space is acquired by interfering with competitors. Strategies such as allelopathy—the direct inhibition of one species by another using noxious or toxic chemicals[93]—and overgrowth resulting from superior growth rates of one species relative to another are the most effective among soft corals. Other strategies used by sessile invertebrates include the use of specialized long tentacles called sweeper tentacles[94] which reach out to neighbors, sting them with well-developed nematocysts, and then proceed to digest the tissue. A related strategy involves
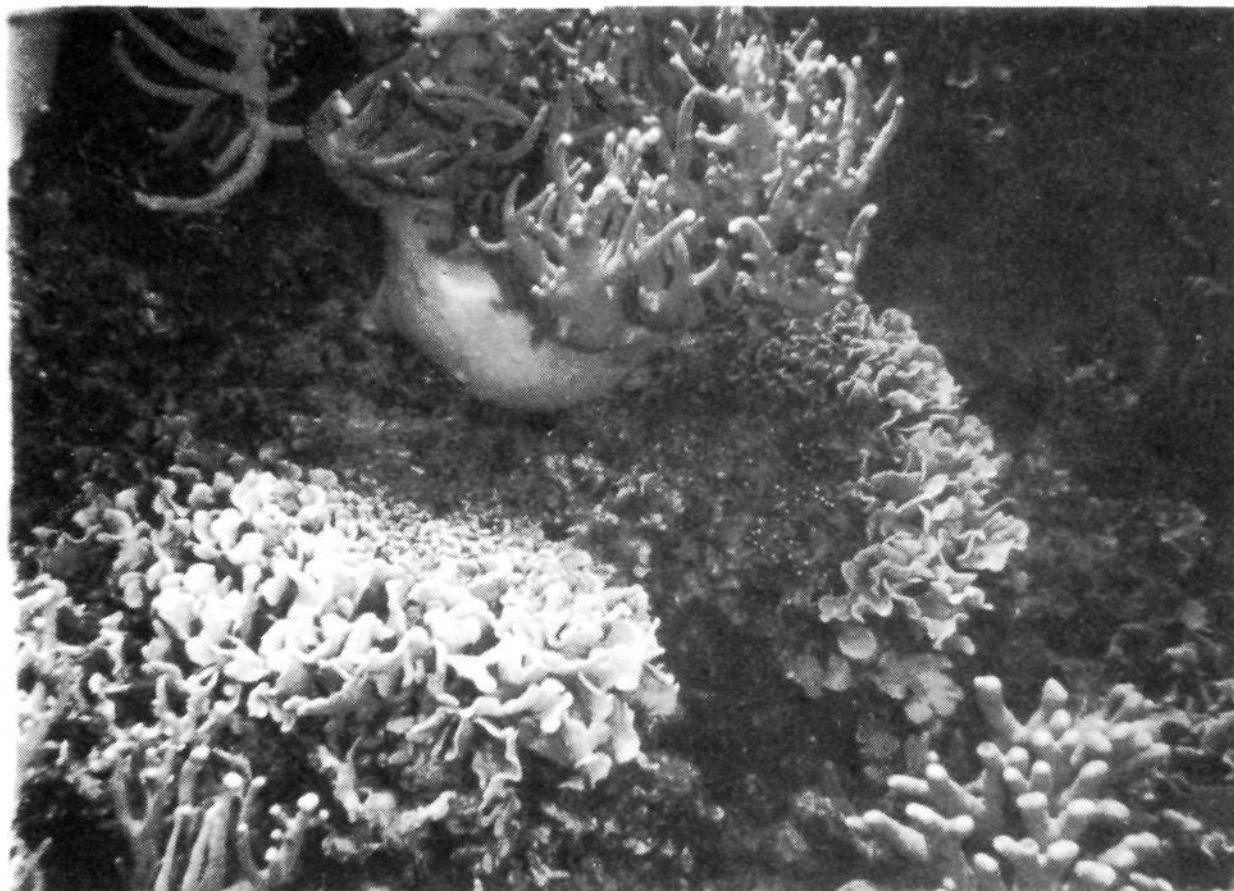
Figure 5. Colony of the soft coral *Sinularia flexibilis* (background) inhibiting the growth of the hard coral, *Pavona cactus* (foreground), in its vicinity (from ref 97; copyright 1983 Springer-Verlag).

the use of mesenterial filaments[95] which are extensions of the gut wall and extruded beyond the colony. The filaments are equipped with proteolytic enzymes and can digest neighboring colonies. The latter strategies are particularly used by scleractinian corals, although recently the octocoral *Briareum stechei*[88] and *B. asbestinum* have been observed to possess sweeper tentacles.[96] Once again this review will focus on *Sinularia flexibilis* and show how it uses its chemical constituents for competitive purposes.

2. Sinularia flexibilis and Allelopathy

As noted earlier, although *Sinularia flexibilis* is quite a soft, fleshy, and apparently defenseless colony, it is a most effective competitor for space on a reef. Bette Willis was studying the growth of the hard coral *Pavona cactus* on a fringing reef in the Palm Islands some years ago. She noted the presence of a colony of *S. flexibilis* growing in the study area, and as time went on, she realized that the soft coral was becoming much larger and that the *P. cactus* colonies in its vicinity were declining. From photographs (Figure 5), it appeared that the growth of *P. cactus* near *S. flexibilis* was stopped, and some parts of the colony appeared dead. As one moved away from the *S. flexibilis* colony, there was a gradation of growth with the normal colony growth form of *P. cactus* starting only beyond 30 cm from the *S. flexibilis* colony. It appeared as if *S. flexibilis* was releasing a "cloud" of toxins which inhibited the growth of *P. cactus* where it fell. This observation, and the hypothesis derived from it, required experimental verification.

In order to verify this hypothesis and to confirm the cause and effect relationship suggested by the field observation, the following field experiments were undertaken. Firstly, we established an elaborate ecological experiment in which we relocated *Sinularia* colonies close to, and within, stands of two different scleractinian corals, *Acropora formosa* and *Porites andrewsi* (cf.

cylindrica). The experiments were carried out in the field and showed that the sinularian coral caused significant necrosis of the hard corals in its vicinity.[97] The second experiment involved the sampling of sea water from around colonies of *S. flexibilis* in situ i.e. on the reef flat using a submersible sampling apparatus[98] (Figure 6). The device consisted of an acrylic enclosure which covered the coral that was open at the base. A submersible bilge pump drew water from around the coral, and pumped it onto reversed-phase Sep-Pak's underwater. The Sep-Pak's collected any organic molecules present in the sea water. They were eluted and shown by TLC to be flexibilide (**21**), dihydroflexibilide (**22**), and a trace of sinulariolide (**20**) in the water surrounding the *S. flexibilis* colonies. The concentration of the major components (**21** and **22**) was between 1–5 ppm. Clearly the "allelopathy" concept now had considerable credibility. Relocation of soft corals into stands of hard corals produced necrosis in hard corals, and diterpenes were detected in the sea water surrounding colonies of *Sinularia flexibilis*.

3. Laboratory Studies

Subsequent experiments showed that flexibilide (**21**) killed hard coral colonies at 5–10 ppm over an 8-h period.[99] Sublethal concentrations of flexibilide (1–5 ppm) caused a sequential expulsion of live zooxanthellae from *Acropora formosa* and *Porites andrewsi*, causing colony bleaching and preventing photosynthesis. This was followed by extrusion of functional nematocysts, the stinging cells of corals, thus preventing food capture. The final effect was a significant reduction in polypal activity as a prelude to eventual death of the hard coral. In total, the effects of even sublethal concentrations of flexibilide (<5 ppm) reduced the fitness of nearby hard corals. In real life, their growth is inhibited, and regions of the hard coral colonies near *Sinularia flexibilis* suffer necrosis and part of the colony dies back. Parallel ex-
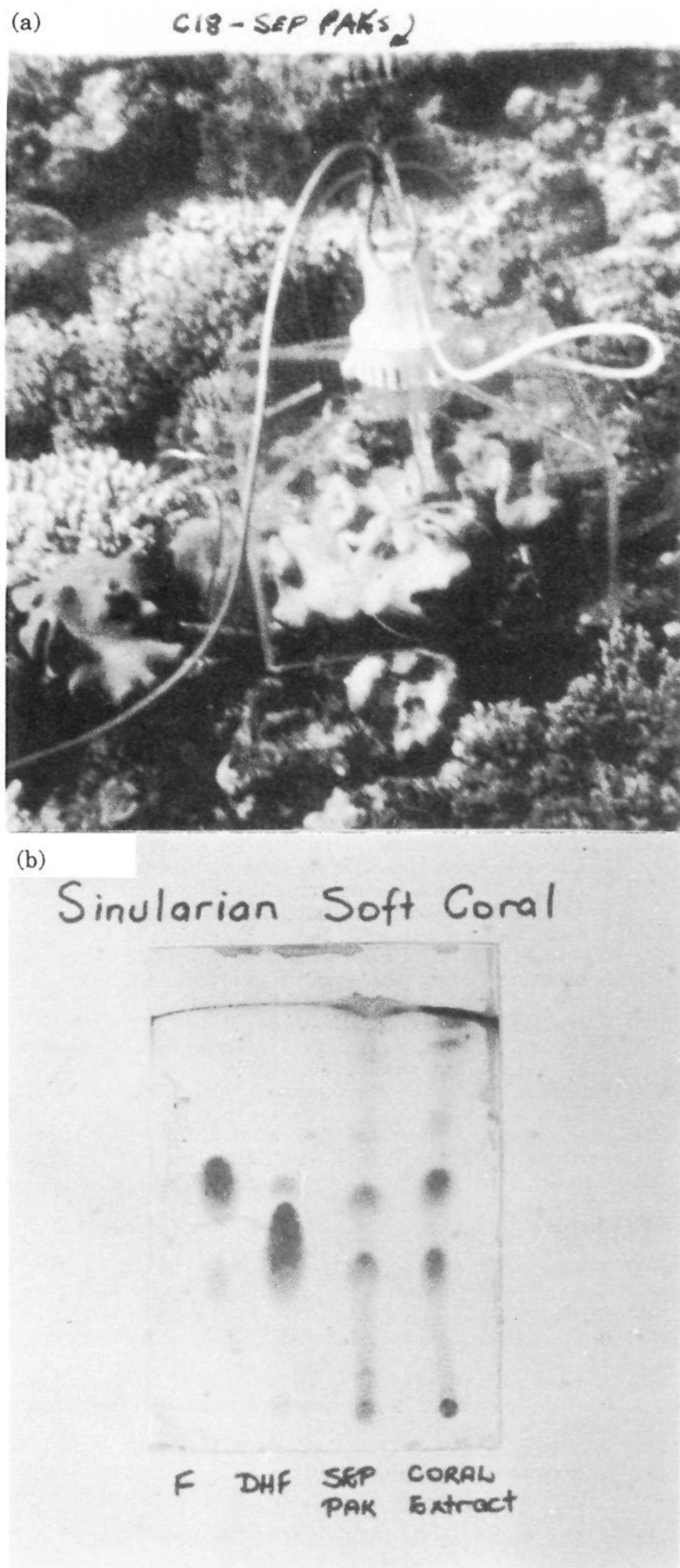
(a)

(b)

Figure 6. Submersible sampling apparatus consisting of an acrylic enclosure and submersible bilge pump which draws water from around a soft coral [(a) *Sarcophyton* sp.] and pumps it through C18 Sep-pak's. The Sep-Paks are eluted and examined by TLC [(b) for *Sinularia flexibilus*: F, flexibilide (**21**); DHF, dehydroflexibilide (**22**). ^{1}H NMR was used to confirm the assignment.[98]]

periments with sinulariolide (**20**) surprisingly showed that it was not effective in this role.

In a related study,[101] it was found that sublethal doses of flexibilide reduce photosynthetic activity of zooxanthellae harvested from *Acropora formosa* and within branches of *A. formosa*, with subsequent demand for an increased rate of respiration. Clearly flexibilide (**21**) is a potent vector in allelopathy.

4. Soft Corals vs Soft Corals

Sinularia flexibilis was also effective in competition between different species of soft corals. In a series of field experiments, the bases of two soft coral species were cemented in plastic flower pots underwater, so that the two colonies were initially in contact. In all cases, initial interspecific necrosis was observed caused by contact with *S. flexibilis* but then somewhat surprisingly, the soft corals moved apart so that contact was no longer possible.[102] We have studied the phenomenon of soft coral movement in some detail for *Nephthea brassica*,[103] and *S. flexibilis* utilizes a similar strategy (Figure 3).

In summary, *Sinularia flexibilis* is able to control the growth of competitors by allelopathy, but if it meets a coral with superior competitive abilities, it is able to move away from the problem. Most recent studies in our group have shown that not only does *S. flexibilis* compete with mature colonies of scleractinian and alcyonacean corals, it also reduces the amount of settlement of juvenile coral spat of other species in its vicinity.[103]

5. Antifouling and Algicidal Studies

Sinularia flexibilis thus uses its potent cocktail of chemicals to maintain and expand the area of solid substrata available for its colonization. The implications of these studies for antifouling applications make *S. flexibilis* metabolites very strong contenders for natural antifouling applications.[105] Indeed, if aqueous extracts of *S. flexibilis* are placed in a special container with a section of coral as a lid, settlement of algae and other fouling organisms on this coral surface is significantly reduced over sea water controls.[106] Further research into the nature of the active antifoulants is in progress. Studies by Tursch[24] have shown that sinulariolide (**20**) is a key chemical in the control of growth by a number of marine algae in laboratory experiments. Other diterpenes have also been shown to be effective antialgal compounds.[107]

In summary, it appears that one or more diterpenes in *Sinularia flexibilis*, especially toxin **24** is responsible for ichthyotoxicity. Flexibilide (**21**) causes necrotic effects in neighbors and is therefore the competitive vector. Sinulariolide (**20**) is an algicidal molecule, presumably responsible for antifouling properties. Examination of the structures reveals that the molecules could be thought of as sharing a common intermediate: Enzymic interconversion could permit responses to different environmental stimulae or challenges (Figure 4).

D. Chemical Aspects of Coral Reproduction: Sinularian Corals

In 1975, Paul Scheuer reported the isolation of a novel, highly oxygenated diterpene which he called pukalide (**26**) from the soft coral *Sinularia abrupta*.[108] Over the next few years, a number of researchers in his group attempted to obtain more pukalide for bioassay purposes, but were unsuccessful. In 1983, we were involved in a study of the spawning of alcyonacean soft corals including a colony of *Sinularia gibberosum* (Tixier-Durivault). We sampled the colony three weeks prior to spawning, and collected eggs at the time of the

annual spawning. To our surprise, the eggs contained pukalide (26) (23 mg/2.4 g) and epoxypukalide (27) (14 mg/2.4 g). The prespawning colony contained neither metabolite at detectible levels. We initially assumed that we had somehow confused the samples, and repeated the collection the following year with identical results. Pukalide (26) and epoxypukalide (27) were synthesized only in the last weeks prior to spawning, and the eggs were the only cells which contained the metabolites. This observation helped to explain the inability of Scheuer's group to recollect pukalide. Pukalide and epoxypukalide are similar to the gorgonian-derived compound lophotoxin (28),[109] although they do not have nearly the potency of this compound. Another sinularian coral, S. polydactyla contained pukalide and a range of derivatives lacking the free aldehyde function of lophotoxin.[110]

Examination of the eggs derived from four different Sinularia species, revealed that they all contained pukalide (26) or epoxypukalide (27).[111] It appears that pukalide and epoxypukalide play some role in the spawning process and may also serve a role in chemical protection of the eggs. Recent research suggests that pukalide induces contractions in the smooth muscles of coral polyps and thus may be involved in the process of expulsion of the eggs from the polyp.[112] Pukalide has been shown to be potent inhibitors of the nicotinic response in adrenal chromaffin cells[113] and appears to induce vomiting in fish.[114] Pukalide and epoxypukalide are present in trace amounts in Leptogorgia virgulata, where they have been shown to be effective in inhibiting the settlement of barnacle larvae of Balanus amphitrite.[82]

E. Concluding Remarks

The chemical constituents of soft corals of the genus Sinularia and especially S. flexibilis have been shown to play a very important role in the survival of the soft corals in their natural environment. Although soft corals and their metabolites have the potential to provide molecules of use in pharmacology[115] and as antifouling agents, their most important application is surely by the soft coral in its interactions with other species. As more chemists show an interest in the way organic molecules interact within nature, our knowledge of our natural environment will be greatly enhanced.

VI. Acknowledgments

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