MARINE SOFT CORALS OF THE GENUS *PSEUDOPTEROGORGIA*: A RESOURCE FOR NOVEL ANTI-INFLAMMATORY DITERPENOIDS[1]

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ABSTRACT.—This paper is a brief review of the diterpenoid chemistry of gorgonian corals of the genus *Pseudopterogorgia*. Previously described compounds and several new diterpenoids are presented in the context of their potential utilization in the development of anti-inflammatory and analgesic drugs.

The marine soft corals (subclass Octocorallia) known as sea whips, sea fans, or sea plumes (also known as gorgonian corals, order Gorgonacea, phylum Cnidaria) are conspicuous members of most tropical and subtropical marine habitats. In the warm waters of the Caribbean Sea gorgonian soft corals are the most abundant octocorals, representing an estimated 38% of the known fauna with over 195 species documented from two major families (1).

Studies of the natural product chemistry of this interesting group of marine invertebrates began in the late 1960s with studies of the more common Caribbean species, and such studies illustrated that the gorgonians contained high levels of secondary metabolites. Since these early investigations, numerous studies of the natural product chemistry of gorgonian corals have been reported. These animals are now recognized to produce acetogenins, sesquiterpenoids, diterpenoids, and, in some cases, highly functionalized steroids. These studies have been summarized by Faulkner (2-4).

Among the early investigations was the remarkable discovery by Weinheimer and Spraggins in 1969 that the Caribbean gorgonian *Plexaura homomalla* contained, in as much as 3% dry weight, a mixture of prostaglandins dominated by the 15-acetate-methyl ester of 15-*epi*-PGA$_2$ (5). Although this interesting discovery brought considerable attention to *P. homomalla*, particularly as it then related to the isolation of commercial quantities of prostaglandins for research purposes, the discovery was not followed by a comprehensive assessment of the biomedical potential of this unique, faunal resource.

In the subsequent years beginning in the early 1970s, my research group has maintained a continuing interest in the chemistry of Caribbean gorgonians, particularly in connection with the adaptations of this group in chemical defense. Although this research has been slow, we have provided evidence in recent investigations that a significant percentage of Caribbean gorgonians contain secondary metabolites which function effectively to deter carnivorous predators (6).

Since the early 1970s, we have also maintained a strong interest in the applications of these defensive metabolites as prototype molecules in the development of new therapeutic agents. This interest was based primarily on the observation that gorgonian metabolites possessed novel structures which are largely unknown from terrestrial sources. In 1980, our biomedical research was established through the California Sea Grant Program as a collaboration with Professor Robert Jacobs, University of California, Santa Barbara. Our studies have focused subsequently upon several areas of pharmacological study, including investigations of the primary pharmacological

mechanisms of action of cell division inhibitors and neuromuscular toxins. More re-
cently, however, our efforts have been primarily focused upon the processes involved in
inflammation and resultant pain. The goals of this collaborative research have been to
probe the fundamentals of inflammation utilizing new, structurally novel, anti-inflam-
matory agents isolated from marine sources. Of course, an applied goal of this research
is to provide the pharmaceutical industry with new structural leads that will ultimately
result in improved anti-inflammatory/analgesic drugs.

In this short paper, I will attempt to review our research aimed at the isolation of
novel anti-inflammatory agents from Caribbean gorgonian corals. I will focus upon our
research with one group, the taxonomically complex gorgonians of the genus *Pseudo-
pterogorgia* Kukenthal. This is justified because these animals have been exceptionally
productive sources for new and novel structure types. I wish to emphasize that the work
summarized here is the product of a group effort with essential contributions in chemis-
try made by my former students, Dr. Sally A. Look, Mr. Mark T. Burch, and Dr.
Maury Bandurraga, and by our collaborators in X-ray methods guided by Professor Jon
Clardy at Cornell University. The brief pharmacological references mentioned here are
results provided by Professor Jacobs and his students at the University of California,
Santa Barbara.

NATURAL PRODUCTS CHEMISTRY OF *PSEUDOPTEROGORGIA*.—Gorgonians of
the genus *Pseudopterogorgia* are best characterized as "sea plumes" based upon their large,
highly finely branched (plumose) and physically soft forms. *Pseudopterogorgia* species are
among the most common of the Caribbean species with over 15 species documented (1).
Studies of the natural product chemistry of *Pseudopterogorgia* species began in 1968 with
investigations of the sesquiterpene hydrocarbons from the most common representative
of this genus, *Pseudopterogorgia americana* Gmelin (7). This investigation showed that *P.
americana* contained complex mixtures of sesquiterpene hydrocarbons, some of which
were already known from terrestrial sources. A subsequent investigation of the same
species showed the presence of an unusual betaine, norzooanemonin (8). From the same
species an interesting secosterol, seco-gorgosterol (9), and a strained, sesquiterpenoid
furan of the germacrene class (10) were also reported. In addition, *Pseudopterogorgia
elisabethae* Bayer has been reported to contain a highly hydroxylated cholestane deriva-
tive (11). More recent studies of *Pseudopterogorgia rigida* Bielschowsky and of an unde-
scribed *Pseudopterogorgia* species from the Florida Keys and Bahamas resulted in the iso-
lation of several oxygenated sesquiterpenoids of the bisabolene class (12,13).

Although the early studies of *Pseudopterogorgia* species emphasized the isolation of
steroids and sesquiterpenoids, we now document that the majority of the *Pseudopterogor-
gia* species produce diterpenoids of unusual structure types. Our investigations of the
diterpenoids from this interesting group began with studies of the common Caribbean
sea plume *Pseudopterogorgia acerosa* Pallas. This gorgonian was found to contain major
amounts of a single, structurally-unprecedented diterpenoid, pseudopterolide [1] (Fig-
ure 1). Pseudopterolide was identified by combined chemical and spectroscopic studies,
based largely upon an X-ray investigation of a suitable *p*-bromophenylurethane deriva-
tive (14). Our attention was drawn to this species because extracts were found to inhibit
the synchronous cell division of the fertilized, sea urchin egg. This latter bioassay has
been extensively used by our collaborators as a simple, ship-board assay illustrating a
high degree of selectivity for cytotoxins which act via the inhibition of tubulin
polymerization (15). Pseudopterolide showed $ED_{50}=8$ μg/ml in this assay and was
further noted to produce unique effects. The compound inhibited overall cell division,
but it did not inhibit nuclear division. Similar effects have been observed with the fun-
gal cytotoxins the cytochalasins, which have been utilized as molecular probes to
explore the biochemistry of cellular processes.

1   pseudopterolide

2   R=OH kallolide A
3   R=H kallolide B

4   kallolide C
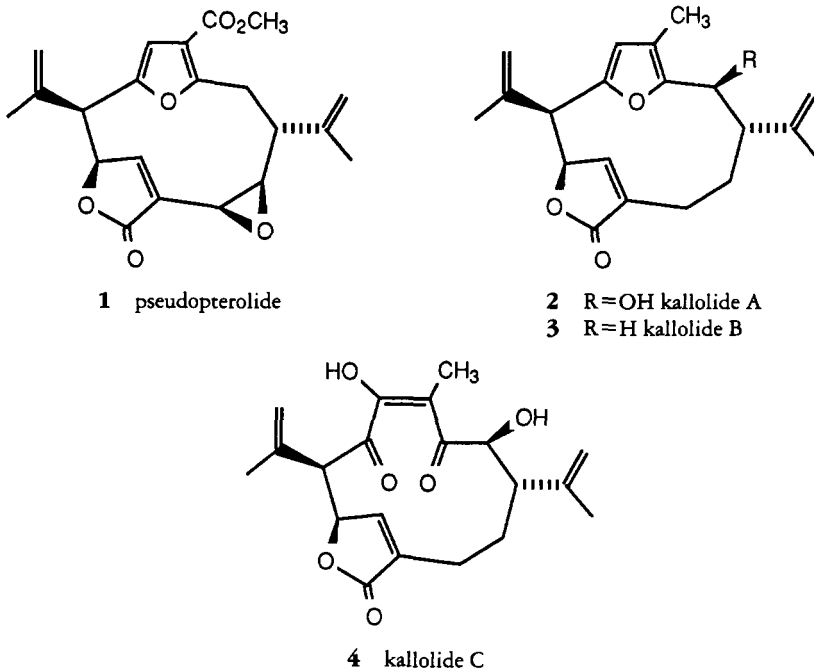
Pseudopterolide was extensively investigated in numerous bioassays, and, in addition to its effects upon cell division, it was found to be a potent inhibitor of topical inflammation. This discovery was significant in that it initiated our efforts to identify new agents in this latter therapeutic area.
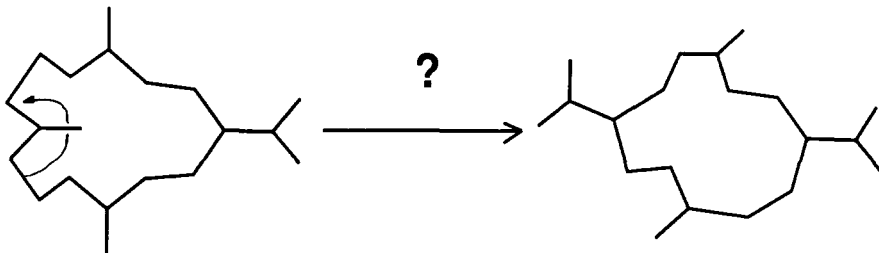

FIGURE 1.   Pseudopterane diterpenoids

More recently, we have discovered several related diterpenoids, the kallolides A-C [2-4], as components of extracts of the related gorgonian *Pseudopterogorgia kallos* Bielschowsky (16). The kallolides possess the identical "pseudopterane" ring system, which is a novel, diterpenoid, carbon skeleton found only in marine systems. Although the biosynthesis of this new class of diterpenoids remains unknown, it would appear reasonable, on structural grounds, that the pseudopterane ring system could be produced from a ring contraction reaction of a cembrene precursor (as illustrated in Figure 1). Derivatives of this ring system have recently been observed in the temperate soft coral *Gersemia rubiformis* (17). Pseudopterolide and several kallolides were found to possess significant topical anti-inflammatory and analgesic activity, comparable in potency to the industrial standard indomethacin (discussed in the next section).

Diterpenoids of the cembrene class are common metabolites of several gorgonian genera, especially *Eunicea* and *Lophogorgia* (18). This is also true for gorgonians of the genus *Pseudopterogorgia* but only in a much more restricted sense. We find that cembrene-based diterpenoids only occur in one or two of the more than 15 species recognized. The major producer of cembrene derivatives is *Pseudopterogorgia bipinnata* Verrill,

a widely distributed member of this genus. *P. bipinnata* produces a large variety of cembrene derivatives somewhat dependent upon collection site and method of extraction. The characteristic of *Pseudopterogorgia* cembrenes is their rather high levels of oxygenation. Two of our recently isolated cembrene derivatives are potent anti-inflammatory agents. In recent, as yet unpublished studies, we have identified cembrenes **5** and **6** (Figure 2) by spectroscopic, X-ray, and chemical methods (19). These compounds are cyclic hemiketals possessing cross-conjugated dieneone functionalities that are potentially derived by hydrolysis of a furanoid precursor. The stereochemistries of these compounds, as determined for one compound by X-ray methods, illustrate the potential of these cembrenes to coordinate metals within the ring and the potential to bind to a wide spectrum of enzyme active sites. More than 15 cembrene derivatives have been isolated from *P. bipinnata*, but **5** and **6** stand out due to their potent anti-inflammatory activities.
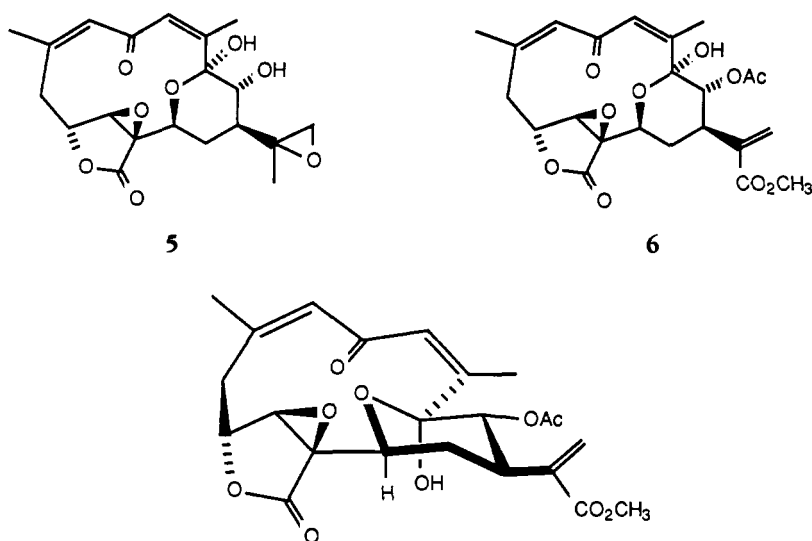

FIGURE 2. Cembrane diterpenoids

Perhaps one of the more interesting discoveries from our group lies in the isolation of diterpene glycosides from several *Pseudopterogorgia* species. While glycosides are common in nature and found in marine organisms primarily as steroidal and triterpenoid glycosides (saponins in echinoderms, for example), gorgonians have only recently been shown to contain this class of natural products. Fusetani and co-workers have recently described terpenoid glycosides from the gorgonian *Euplexaura* sp. and steroidal glycosides from the gorgonian *Anthoplexaura dimorpha* (20,21). Our research with the Pacific gorgonian *Muricea fruticosa* showed this gorgonian to produce steroidal aminogalactose glycosides composed of pregnane derivatives (22).

During investigations of Caribbean based gorgonians on board the University of Miami's research vessel *Calanus*, we encountered the deeper water and finely branched *Pseudopterogorgia elisabethae* in numerous habitats in the Florida Keys and Bahama islands. Tlc analysis of the crude $CHCl_3$ extracts of this animal showed the presence of several more polar compounds in exceptionally high yield. The extracts were consistently highly bioactive, and, subsequently, Sally Look was successful in isolating four closely related glycosides, the pseudopterosins A-D {**7-10**} (Figure 3) (23,24). The pseudopterosins are D-xylose pentosides of an *o*-catechol diterpene of a rare structure class. The pseudopterosins B-D are simple acetate positional isomers, all of which can
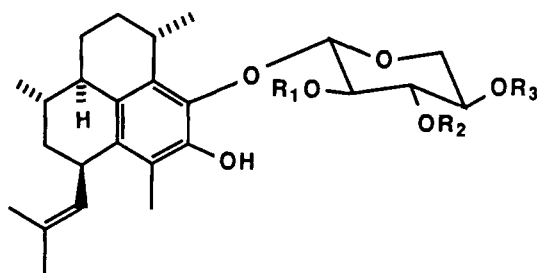
7   $R_1=R_2=R_3=H$ pseudopterosin A
8   $R_1=Ac$, $R_2=R_3=H$ pseudopterosin B
9   $R_2=Ac$, $R_1=R_3=H$ pseudopterosin C
10 $R_3=Ac$, $R_1=R_2=H$ pseudopterosin D
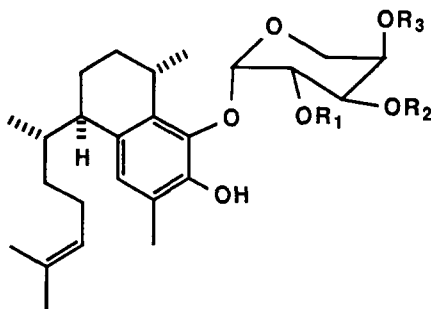
11 $R_1=R_2=R_3=H$ seco-pseudopterosin A
12 $R_1=Ac$, $R_2=R_3=H$ seco-pseudopterosin B
13 $R_2=Ac$, $R_1=R_3=H$ seco-pseudopterosin C
14 $R_3=Ac$, $R_1=R_2=H$ seco-pseudopterosin D

FIGURE 3.   Pseudopterosin and seco-pseudopterosin diterpene glycosides

be converted to a single tetra-acetate derivative with Ac_2O in pyridine or to the un-acetylated compound, pseudopterosin A, by treatment with $LiAlH_4$ in Et_2O.

The structure determinations of the pseudopterosins were based upon an X-ray determination, provided by Professor J. Clardy and his co-workers, of the major crystalline derivative, pseudopterosin C [9].

In separate investigations we have also encountered an apparently undescribed *Pseudopterogorgia* species (25) that contains a related but new class of diterpenoid glycosides. The new compounds, the seco-pseudopterosins A-D [11-14] (Figure 3), are arabinose glycosides of a closely related class of diterpenoids. The fundamental carbon skeleton of the aglycone of the seco-pseudopterosins is identical to the "serratulane" class of diterpenoids encountered in composite plants of the genus *Eremophila* (26). Unlike the pseudopterosins, the seco derivatives were unsuitable for X-ray analysis. Hence, the structure determinations of these compounds relied heavily upon nmr investigations and upon degradative chemical methods. Ultimately, the aglycone from seco-pseudopterosin A was isolated and chemically related to a derivative previously produced from an *Eremophila* metabolite.

The dissimilarities between the pseudopterosin and seco-pseudopterosin classes also include the stereochemistry of their glycoside linkages to their corresponding sugars. In the pseudopterosins the linkage is clearly equatorial or β, while in the seco derivatives the linkage is axial or α. These dissimilarities do not seem to affect the overall biological properties of these compounds, however, as the seco derivatives are qualitatively as active as the pseudopterosins.

ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF *PSEUDOPTEROGORGIA* DITERPENOID METABOLITES.—In assessing the anti-inflammatory properties of *Pseudopterogorgia* metabolites, our collaborators at UC-Santa Barbara have utilized a topical inflammation assay designed to probe the antagonistic effects of marine natural products against the potent inflammatory diterpenoid phorbol myristate acetate (PMA). The assays involve the induction of topical inflammation in the mouse ear by topical application of PMA, followed by treatment with the test compound (the marine natural product) at initial screening doses of 50 μg/ear. The degree of reduction of inflammation is measured relative to a control ear and the values are expressed as percent reduction of edema (inflammation).

In assessing the analgesic effects of *Pseudopterogorgia* diterpenoids, the method of Hendershot and Forsaith (27) was utilized. This standard method involves monitoring the phenylquinone induced stretch-reflex reaction in mice. In both the anti-inflammatory and analgesia assays the commercial anti-inflammatory drug indomethacin was used as a standard to assess relative potency.

Using the above methods, pseudopterolide [1] showed an 83% reduction in inflammation. The kallolides show similar effects. Although a recent discovery and not as yet completely quantified, the hemiketal cembrenes from *P. bipinnata* show anti-inflammatory activities with equivalent potency to indomethacin.

The pseudopterosins and seco-pseudopterosins, because of their structural novelties and potencies, have been given a high priority in subsequent biotesting. The pseudopterosins and seco-pseudopterosins all show reductions of between 75 and 95% in edema at standard testing doses. ED_{50} values for pseudopterosin A were determined to be 8.3 μg/ear. In analgesia the pseudopterosins and seco-pseudopterosins were analogous in potency to indomethacin.

Although the majority of the testing was completed using completely topical methods (induction of inflammation topically followed by treatment of inflammation topically), our collaborators have illustrated that the pseudopterosins possess in vivo activity in the same assay. Induction of inflammation was produced by the standard PMA topical application. However, rather than treatment with pseudopterosins topically, the compound was injected intraperitoneally. Using this protocol, the pseudopterosins were shown to have ED_{50} values of between 10 and 15 mg/kg. Similarly, the phenylquinone assay was performed in the same in vivo mode, resulting in ED_{50} values for the inhibition of writhing of between 4 and 6 mg/kg.

The mechanisms of action of these aforementioned *Pseudopterogorgia* diterpenoids in the inhibition of inflammation and resultant pain remain largely unknown. Our pharmacological collaborators are actively pursuing this question, particularly with the pseudopterosins which are known to possess a unique and as yet undefined mechanism of action.

A BIOLOGICAL RATIONALE FOR THE PRESENCE OF ANTI-INFLAMMATORY DITERPENOIDS IN *PSEUDOPTEROGORGIA*.—The rather high levels and frequency of occurrence of anti-inflammatory compounds in *Pseudopterogorgia* lead to the natural question of their possible functions in nature. My conclusion is that these mammalian, biological properties are fortuitous and that the metabolites in *Pseudopterogorgia* function as defensive compounds in their natural habitat. However, there may be an alternative explanation which can be derived from recent knowledge of the natural product chemistry of marine soft corals in general.

In surveying the literature summarized by Faulkner (2-4), it becomes clear that some species of octocorals contain unusually high levels of structurally unique prostaglandin derivatives. Examples of these compounds are summarized in Figure 4. Examples include the PGA_2 derivatives found in *P. homomalla* (5), PGE derivatives from the

Plexaura homomalla

Lobophytum depressum

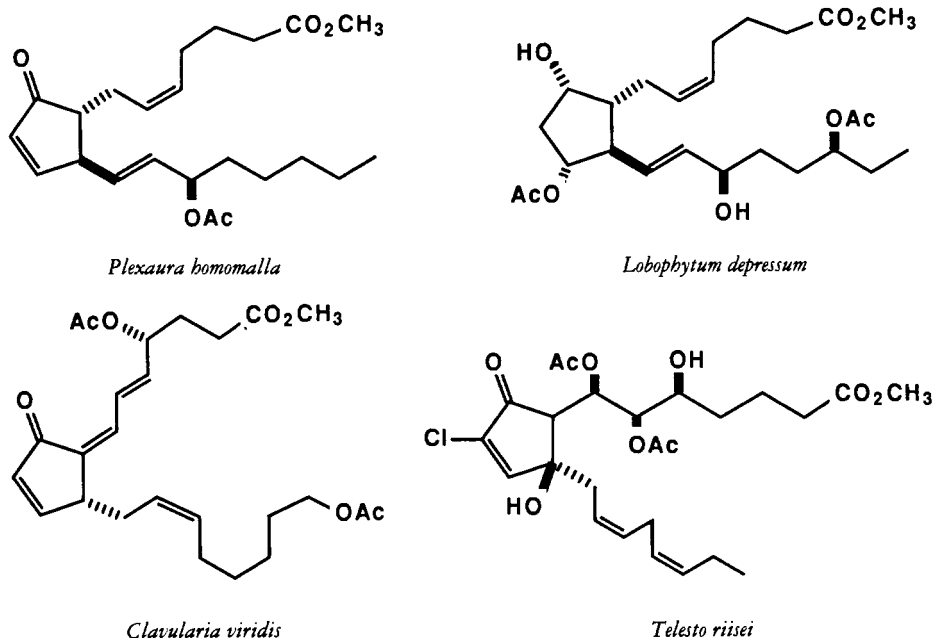*Clavularia viridis*

Telesto riisei

FIGURE 4.   Naturally occurring prostanoids from marine soft corals

Red Sea alcyonacean *Lobophytum depressum* (28), a variety of PGA derivatives from the Indo-Pacific alcyonacean *Clavularia viridis* (29-34), and several chlorinated prostaglandins from the Hawaiian telestacean *Telesto riisei* (35).

The observations of prostaglandin derivatives in these diverse octocorals clearly illustrate that arachidonic acid metabolism is in place in some of these species and is likely to be represented, perhaps in somewhat smaller overall yields, in all octocorals. Although speculative, it is certainly possible that marine octocorals have evolved the synthesis of other metabolites, perhaps diterpenoids, which function to regulate arachidonic acid metabolism. If this is the case, marine invertebrates of this class should provide a rich resource for novel anti-inflammatory agents that would appear to regulate inflammation by unknown, but more importantly, new mechanisms of pharmacological action.

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