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REVIEW PAPER

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Elucidation of the biosynthetic origin of the anti-inflammatory pseudopterosins

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Abstract The pseudopterosins are a family of diterpene glycosides isolated from the gorgonian coral *Pseudopterogorgia elisabethae*. These metabolites exhibit potent anti-inflammatory activity, and this review describes our efforts to elucidate their biosynthetic origin. A radioactivity-guided isolation was used to identify the terpene cyclase product. In addition, a detailed NMR-guided search for potential biosynthetic intermediates identified metabolites which were tested by incubating ^{3}H-labeled analogues with a cell-free extract of the coral. All labeled metabolites were generated biosynthetically, and radiochemical purity was established by a combination of HPLC purification and derivatization. In summary, pseudopterosins are produced by a cyclization of geranylgeranyl diphosphate to elisabethatriene, aromatization to erogorgiaene, two successive oxidations to 7,8-dihydroxyerogorgiaene and a glycosylation to afford a seco-pseudopterosin as a key intermediate. A dehydrogenation leads to amphilectosins which undergo ring closures to yield the pseudopterosins.

Keywords Terpene · Gorgonian coral · Biosynthesis · Anti-inflammatory · Pseudopterosin

Introduction

The field of marine natural products chemistry has enjoyed sustained growth over the past 20 years or more [1]; however, our understanding of the biosynthetic origin of these compounds has not developed at the same pace. The biosynthesis of two classes of marine natural products, polyketides and non-ribosomal peptides, are being examined in a number of marine organisms. This is due to the existence of a high degree of homology of polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs) and is leading to an understanding of the mechanism of production of these compounds. This research is also leading to answers concerning the source of the biosynthetic pathway in the complex assemblages of an invertebrate and its microbial symbionts.

Terpenes represent the largest group of natural products with many notable examples from the marine environment. There has been a very limited amount of experimental work addressing fundamental biosynthetic questions with this group of marine natural products. In 1970, Anderson and coworkers [2] demonstrated the de novo biosynthesis of crassin acetate in the gorgonian, *Pseudoplexaura porosa*. Other examples of terpene biosynthetic investigations in marine organisms include studies on the *Dendrodoris* sp. nudibranchs [3–7]. These experiments revealed that the drimane sesquiterpenes were produced de novo from mevalonate. Additionally, the isonitrile metabolites isolated from various sponges are a family of terpenes that have been extensively investigated. This work confirmed the role of inorganic cyanide in the biosynthesis and showed that the incorporation of cyanide into the compounds is an enzymatic process [8–11].

Gorgonians, also known as sea whips, sea fans or sea plumes, are prominent members of tropical and subtropical habitats world-wide. In the Bahamas and Florida, gorgonians represent an estimated 38% of the known fauna with over 195 species documented from the families Briareidae, Plexauridae and Gorgoniidae [12]. These organisms have proven to be a prolific source of novel bioactive natural products, particularly terpenes which exhibit a range of biomedical activities [13, 14].

The pseudopterosins are a family of diterpene glycosides produced by the gorgonian *Pseudopterogorgia elisabethae* which is found in only a few areas of the Caribbean. Interestingly, different members of the pseudopterosin family are found in *P. elisabethae*

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collected in distinct geographic locations. There are 26 derivatives, pseudopterosins A–Z (PsA–PsZ) and in most cases, the pseudopterosins are present in 2–5% of the crude extract. In spite of the relatively large number of pseudopterosins, there are only minor changes in the structures within this family. All pseudopterosins possess the amphilectane diterpene skeleton; the carbohydrate moiety can be located at either C-9 or C-10, the identity and degree of acetylation can vary and the isobutenyl group at C-1 can be either α or β. Selected pseudopterosins discussed in this review are shown in Fig. 1. Pseudopterosins A–D (**1**–**4**) have been isolated from *P. elisabethae* collected from Grand Bahama Island and the central Bahamas [15], Ps E–J were isolated from collections in Bermuda, and Ps K and L were the sole pseudopterosins of collections from Great Abaco Island [16]. Collections of the closely related *P. kallos* from Cosgrove Shoal near the Marquesas Keys in Florida revealed the presence of seco-pseudopterosins A–D (**7**–**10**, Fig. 1) [17] which possess the serrulatane ring system. The pseudopterosins and seco-pseudopterosins are anti-inflammatory and analgesic agents with potencies superior to that of existing drugs such as indomethacin in mouse ear models [18, 19]. Further, one of the pseudopterosins has a commercial market as a skin care product. They are not active against phospholipase A_2, cyclooxygenase and cytokine release or as regulators of adhesion molecules. Evidence suggests the pseudopterosins block eicosanoid release rather than biosynthesis in murine macrophages [20]. A simple derivative of pseudopterosin A, methopterosin, has successfully completed Phase II clinical trials as a topical anti-inflammatory agent.

Strategy for pathway elucidation

As described in a review by Garson [21], the primary problem in pathway elucidation work is the very low levels of incorporation of labeled metabolites thus "forcing" researchers to use radioisotopes rather than stable isotope methodology. In an effort to identify plausible intermediates involved in pseudopterosin biosynthesis, our strategy was to examine the terpene chemistry of *P. elisabethae* from various geographic locations. Collections of this gorgonian from the Florida Keys indicated that such populations contain a low concentration of pseudopterosins but have a great variety of serrulatane diterpenes. Conversely, extracts of *P. elisabethae* from the Bahamas have higher concentrations of pseudopterosins, but a much lower diversity of diterpenes. LC-MS was used to evaluate extracts for general terpene diversity and GC-MS was the instrument of choice for examining hydrocarbon fractions for potential terpene cyclase products.

As discussed above, in order to complete the pathway elucidation of the pseudopterosins, radiolabeled precursors were necessary to provide sufficient sensitivity. [C_1–^{3}H]-Geranylgeranyl diphosphate (GGPP) is commercially available and, through incubations with cell-free extracts (CFEs) allowed us to produce ^{3}H-labeled intermediates by employing biosynthetic methods. In order to provide a high level of confidence in our labeling studies, radiochemical purity of precursors and products was rigorously established by (1) HPLC purification of the natural product, (2) derivatization, and (3) HPLC purification of the derivative. If the specific

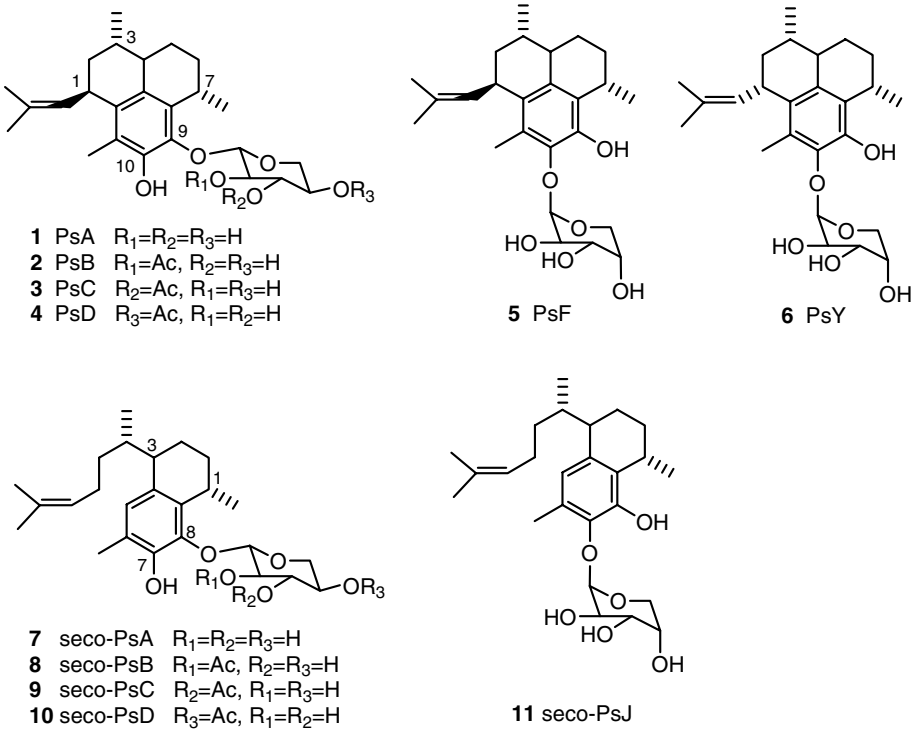


Fig. 1 Structures of selected pseudopterosins and seco-pseudopterosins

activity of the natural product and the derivative were similar then the recovered metabolite was judged to be radiochemically pure.

In 2003, we presented a proposed biosynthetic origin of the pseudopterosins based on a number of structurally related molecules isolated from *P. elisabethae* [22]. Since then, we have conducted numerous biosynthetic experiments with labeled precursors and rigorously demonstrated radiochemical purity in each instance. This review will summarize these experiments and provide an overview of our revised pathway leading to the pseudopterosins.

Development of conditions to evaluate potential biosynthetic intermediates

Given the paucity of literature discussing biosynthetic experiments of natural products from gorgonian corals, the first task in elucidating the biosynthesis of the pseudopterosins was to establish conditions in which to evaluate potential intermediates. A viable CFE of *P. elisabethae* was generated using conditions derived from the plant biosynthesis literature [23]. The CFE was prepared by homogenizing flash frozen gorgonian tissue in a phosphate buffer containing EDTA and β-mercaptoethanol at the pH of the organism (7.7) with liquid nitrogen. Cellular debris was removed by centrifugation and the supernatant stored in aliquots at −80 °C for use in biosynthetic experiments. The addition of protease inhibitors did not improve biosynthetic activity. In a typical experiment, ca. 35,000 dpm ^{3}H-labeled pseudopterosins was recovered from an incubation of 1 μCi ^{3}H-GGPP with 25 mL CFE.

Conditions were also established to examine the metabolism of labeled precursors with live specimens of *P. elisabethae* [23]. Freshly collected gorgonians were placed in separate plastic bins with the minimal amount of sea water to cover the organism and aerated. To help ensure that water-soluble precursors would be ingested by the filter feeding gorgonian, labeled precursors were adsorbed onto a small amount of invertebrate food before addition to the seawater. The organisms were maintained in the minimal amount of seawater for 24 h to maximize uptake of the precursor. They were then returned to the ocean for an additional 72 h to allow for the metabolism of the precursors in their natural environment. Under these conditions, both ^{3}H-GGPP and ^{14}C-xylose were efficiently transformed to the pseudopterosins [23].

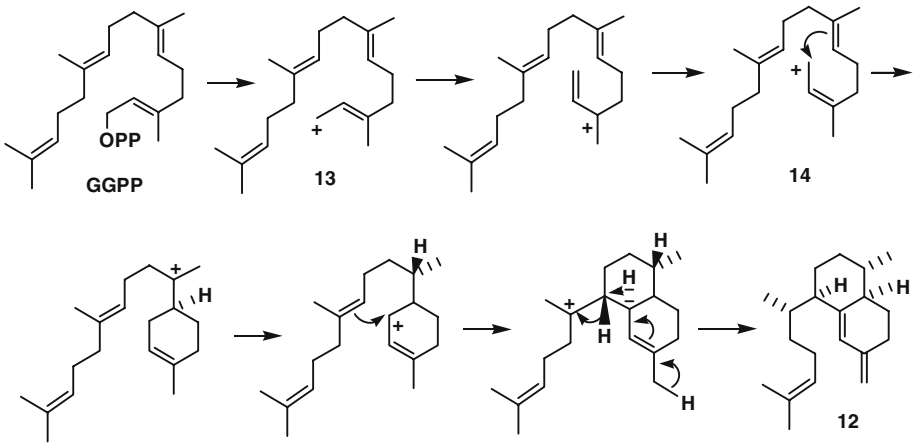
Identification of elisabethatriene, the diterpene cyclase product with a serrulatane skeleton

One of the key intermediates in terpene biosynthesis is the cyclase product. In terrestrial systems, numerous such intermediates have been characterized and, in general, these compounds are unsaturated hydrocarbons. Terpene synthases (= terpene cyclases) catalyze intramolecular, electrophillic reactions with acyclic intermediates at various metabolic branch points in isoprenoid metabolism [24]. A typical cyclization reaction initiates when the prenyl diphosphate substrate is ionized, and subsequently an intramolecular attack by a double bond of the prenyl chain at C-1 occurs to generate a cyclic carbocationic intermediate. Following this, succeeding steps may involve ring formation by internal additions via other double bonds, hydride shifts, methyl migrations, and Wagner-Meerwein rearrangements before termination of the reaction by capture of a nucleophile (such as water) to yield an alcohol or deprotonation to yield a hydrocarbon [25]. A growing number of terpene synthases in the monoterpene, sesquiterpene and diterpene series have been isolated and cloned from plant and, more recently, microbial sources [e.g., 26–30]. The lack of sequence homology in terpene synthases precludes the direct molecular approach to investigations of this fundamental step in terpene biosynthesis [31].

In 2000, we reported the structure of the diterpene cyclase product leading to the pseudopterosins which represented the first such intermediate identified from any marine organism [32]. A radioactivity-guided isolation strategy was employed as a direct method of identifying the cyclase product. We reasoned that we could gain rapid access to the diterpene cyclase product by isolating the least polar radioactive intermediate following an incubation of a CFE with ^{3}H-GGPP. This proved to be a worthwhile approach. The hexane fraction from the quenched incubation mixture was subjected to normal phase HPLC and the first radioactive peak collected and further purified by reversed phase HPLC with monitoring of radioactivity. Elisabethatriene (**12**) was shown to be the only radioactive compound in this HPLC analysis. Elisabethatriene was found to be an unsaturated C-20 hydrocarbon as expected, however, NMR analysis indicated that this cyclase product possessed the serrulatane skeleton rather than the anticipated amphilectane skeleton of the pseudopterosins. This is surprising, as in most cases the terpene cyclase product has the same carbon skeleton as the fully functionalized terpene. This added an element of novelty to this first pathway elucidation project of a marine terpene, and clearly a key feature of the pathway would be the nature of the formation of the third ring of the pseudopterosins. Radiochemical purity of elisabethatriene was established by rigorous purification by HPLC, derivatization to an endocyclic diene, and subsequent HPLC purification of the derivative with comparison of specific activity [32].

While detailed mechanistic studies to elucidate the precise mechanism of cyclization of GGPP to elisabethatriene have not been conducted, the formation of **12** presumably proceeds either as shown in Scheme 1 or in Scheme 2. In Scheme 1, the allylic cation **13** derived from GGPP undergoes an *E–Z* isomerization (common in terpene cyclizations) followed by a ring closure to

Scheme 1 Possible mechanism of formation of elisabethatriene (pathway A)



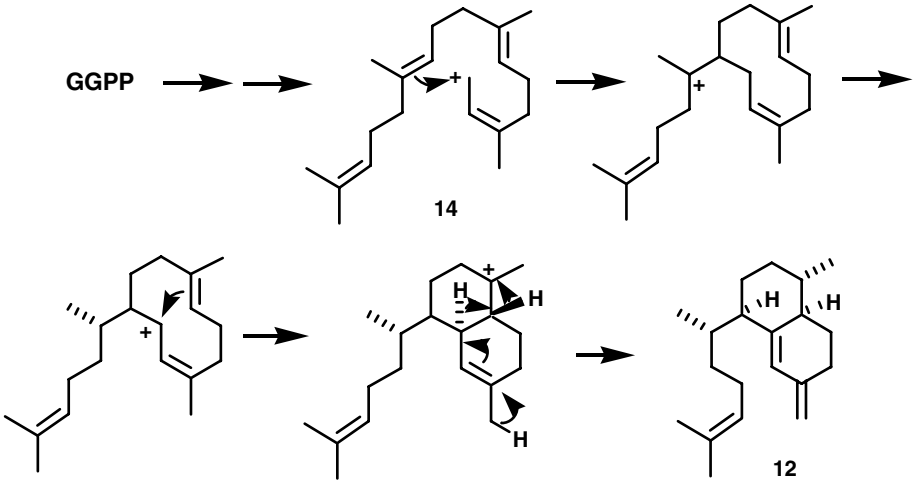
generate a six-membered ring. Rearrangement of the tertiary carbocation to an allylic cation facilitates the second ring closure. Proton abstraction and subsequent rearrangement then leads to elisabethatriene (**12**). Scheme 2 provides an alternative route to elisabethatriene starting with intermediate **14**. In this case, rather than the six-membered ring being produced first, a ten-membered ring is initially constructed. Hydride migrations then lead to the bicyclic ring system which undergoes proton abstraction and rearrangement as above to afford elisabethatriene (**12**).

Oxidations of elisabethatriene

It was evident that the transformation of elisabethatriene (**12**) to pseudopterosins required a dehydrogenation/aromatization process. Erogorgiaene (**15**) was initially reported from a collection of *P. elisabethae* from Columbia [33] and thus seemed like a plausible intermediate in pseudopterosin biosynthesis. We isolated **15** from our collections of *P. elisabethae* from the Florida Keys, however it was not present in isolable quantities in our Bahamian collections of this coral. Both elisabethatriene (**12**) and erogorgiaene (**15**) were produced in ^{3}H-labeled form using incubations of a CFE with ^{3}H-GGPP [34]. Incubation of **12** (300,000 dpm) with a CFE resulted in the production of ^{3}H-labeled **15** (2,060 dpm). Radiochemical purity of erogorgiaene was established by HPLC purification, derivatization to its epoxide and subsequent HPLC purification of the derivative. There was no significant change in the specific activity of the derivative, thus confirming the conversion of elisabethatriene to erogorgiaene. To test for the conversion of **15** to the pseudopterosins, ^{3}H-labeled **15** (5,130 dpm) was incubated with a CFE of *P. elisabethae* and pseudopterosins A–D purified by HPLC. Liquid scintillation counting indicated that the pseudopterosins were radioactive and, in fact, there was a 5.7% radiochemical yield for this transformation [34].

It was clear from the structures of elisabethatriene (**12**) and its aromatized product erogorgiaene (**15**) that mono- and dihydroxy derivatives of **15** were likely intermediates in pseudopterosin biosynthesis [35]. We examined our *P. elisabethae* collections from the Florida Keys as a potential source of such compounds as we had found this to be the source of much greater diterpene diversity than collections from other sites. 7-Hydroxy

Scheme 2 Possible mechanism of formation of elisabethatriene (pathway B)



erogorgiaene (**16**) was isolated as a minor component of this extract and the 8-hydroxy isomer was not found, suggesting that the first oxidation occurs at C-7 of erogorgiaene. 7,8-Dihydroxyerogorgiaene (**17**) initially proved difficult to isolate and thus a standard sample was synthesized by a hydrolysis of seco-pseudopterosin J (**11**) with methanolic HCl. Using this authentic standard to guide the isolation, catechol **17** was purified from the *P. elisabethae* extract by TLC followed by repeated reversed phase HPLC.

The presence of 7-hydroxyerogorgiaene (**16**) and 7,8-dihydroxyerogorgiaene (**17**) from an extract of *P. elisabethae* suggested that these were intermediates in pseudopterosin biosynthesis. To experimentally test this hypothesis, radiolabeled **15**, **16** and **17** were prepared, incubated with a CFE of *P. elisabethae* and the production of labeled pseudopterosins monitored by scintillation counting. From an incubation with ^{3}H-GGPP, erogorgiaene and 7-hydroxyerogorgiaene were both isolated with sufficient radioactivity for subsequent incubations [35]. Due to the low natural abundance of 7,8-dihydroxyerogorgiaene (**17**) and the relatively high abundance of seco-pseudopterosin J (**11**) in the cell free extract, ^{3}H-labeled diol **17** was produced from the hydrolysis of the glycoside (**11**).

In order to confirm radiochemical purity of these three metabolites, each was derivatized and the derivatives purified by HPLC. Erogorgiaene (**15**) was transformed to its epoxide which was purified and shown to have the same specific activity as the isolated erogorgiaene. 7-Hydroxyerogorgiaene (**16**) was transformed to its 7-methoxy derivative and 7,8-dihydroxyerogorgiaene (**17**) transformed to its dimethoxy derivative. In both cases, the specific activity of the methoxy derivatives was the same as that of the isolated alcohols [35]. To confirm the intermediacy of **16** and **17** in pseudopterosin biosynthesis, a series of incubations was performed. Firstly, the conversion of 7-hydroxyerogorgiaene (**16**) to 7,8-dihydroxyerogorgiaene (**17**) was confirmed by incubating **16** (297,400 dpm) with a CFE. This resulted in the production of radioactive 7,8-dihydroxyerogorgiaene (**17**, 3,460 dpm) which was shown to be radiochemically pure by derivatization. Labeled **16** and **17** were both transformed to pseudopterosins A–D by a CFE of *P. elisabethae*. In both cases, radiochemical purity was established by hydrolyzing a mixture of Ps A, B, C and D to Ps A and monitoring the specific activity [35].

Amphilectosins as key biosynthetic intermediates

The confirmation of elisabethatriene (**12**), erogorgiaene (**15**), 7-hydroxyerogorgiaene (**16**) and 7,8-dihydroxyerogorgiaene (**17**) as intermediates in pseudopterosin biosynthesis indicated that the ring closure of the serrulatane skeleton to the amphilectane ring system must occur relatively late in the biosynthetic pathway. We had observed that seco-pseudopterosins co-occur with pseudopterosins in many of our collections of *P. elisabethae*. Together with the biosynthetic data discussed above, this suggested that seco-pseudopterosins may be biosynthetic precursors of the pseudopterosins. To experimentally test this hypothesis, ^{3}H-labeled seco-pseudopterosin J (**11**) was prepared by biosynthetic means and radiochemical purity established by derivatization to its dihydro analogue and monitoring of specific activity [36]. Incubation of ^{3}H-labeled seco-pseudopterosin J (**11**) with a CFE resulted in the production of radioactive pseudopterosins F (**5**) and Y (**6**). In this case, the derivatization reaction to establish radiochemical purity was the hydrolysis of the pseudopterosins to their aglycones.

Confirmation of the involvement of seco-pseudopterosins in pseudopterosin biosynthesis implied the existence of a seco-pseudopterosin derivative with a functionality at C-13 to facilitate a ring closure. We therefore conducted an NMR-guided examination of the mid-polarity extract of *P. elisabethae* from the Florida Keys to search for such compounds. This resulted in the isolation of two new compounds which we termed amphilectosins A and B (since these provide the key intermediate in the formation of amphilectane diterpenes) [36]. These new compounds (**18** and **19**, respectively) contained a diene moiety in the side chain thus suggesting that the ring closure could occur by a nucleophilic attack of the aromatic ring at C-12–C-13 double bond. The intermediacy of the amphilectosins was confirmed by incubating radioactive **18** and **19** with CFEs and monitoring for the production of ^{3}H-labeled pseudopterosins. Amphilectosin A (**18**) was transformed to pseudopterosin Y (**6**) and amphilectosin B (**19**) was transformed to pseudopterosin F (**5**). Radiochemical purity was established by acid catalyzed ring closure/hydrolysis of the amphilectosins to the corresponding pseudopterosin aglycones and monitoring specific activity. These biosynthetic experiments revealed that the origin of the α- and β-isobutenyl groups is due to the selective ring closure of the *cis*- and *trans*-amphilectosins.

The acetylation of the carbohydrate moiety of the pseudopterosins appears to be the final step in this biosynthetic pathway. In incorporation experiments with ^{3}H-GGPP, pseudopterosin A had a significantly higher specific activity that pseudopterosins B–D with both in vitro and in vivo conditions [23]. This higher specific activity indicates that this is an earlier intermediate in the pathway than ones with lower specific activity. Thus, the completed biosynthesis of the pseudopterosins is as outlined in Scheme 3.

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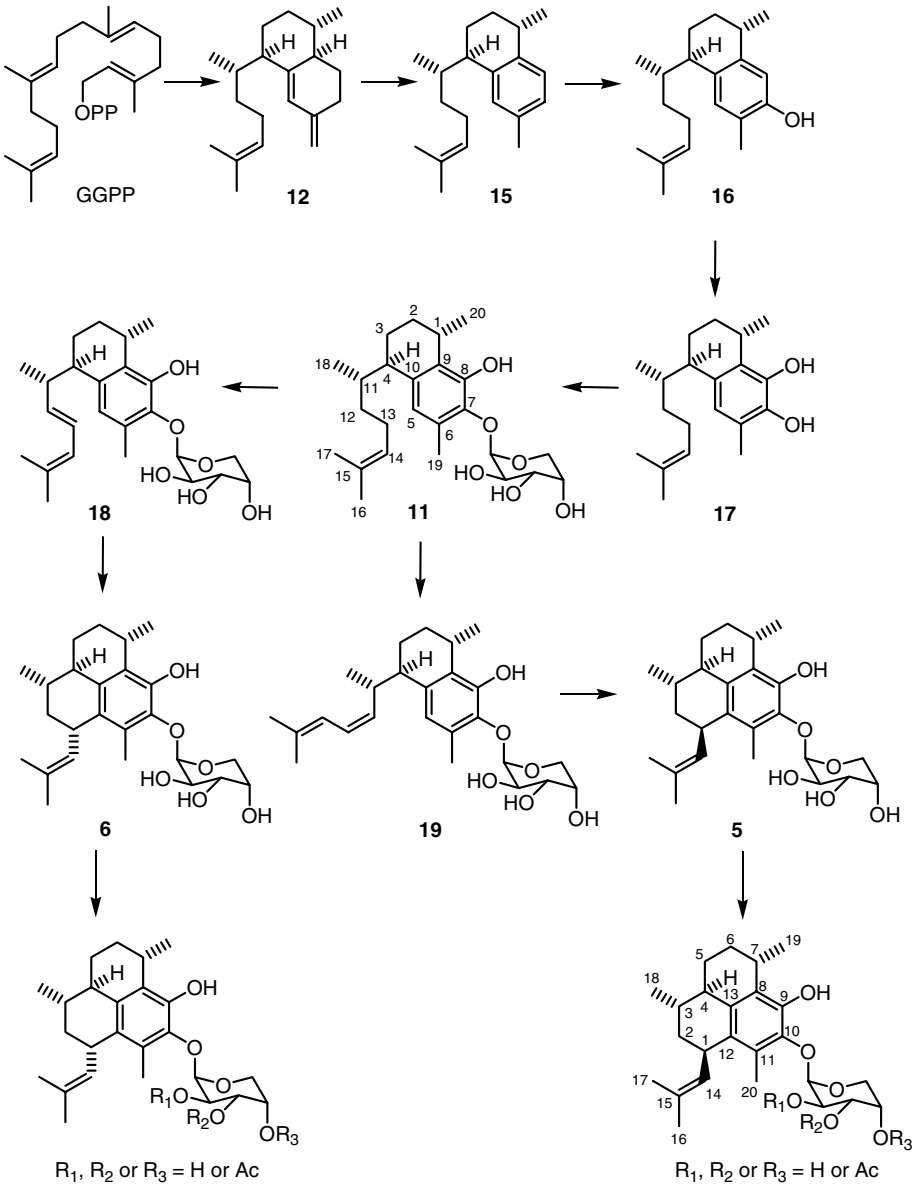
Scheme 3 Pseudopterosin biosynthesis



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References

1. Faulkner, DJ (2002) Marine Natural Products. Nat Prod Rep 91:1–48 and previous reviews in this series
2. Rice JR, Papastephanou C, Anderson DG (1970) Isolation, localization, and biosynthesis of crassin acetate in *Pseudoplexaura porosa*. Biol Bull 138:334–343
3. Cimino G, De Rosa S, De Stefano S, Sodano G, Villani G (1983) Dorid nudibranch elaborates its own chemical defense. Science 219:1237–1238
4. Cimino G, De Rosa S, De Stefano S, Morrone R, Sodano G (1985) The chemical defense of nudibranch molluscs. Structure, biosynthetic origin and defensive properties of terpenoids from the dorid nudibranch *Dendrodoris grandiflora*. Tetrahedron 41:1093–2000
5. Cimino G, De Rosa S, De Stefano S, Sodano G (1985) Observations on the toxicity and metabolic relationships of polygodial, the chemical defense of the nudibranch *Dendrodoris limbata*. Experientia 41:1335–1336
6. Cimino G, De Rosa S, De Stefano S, Sodano G (1986) Marine natural products: new results from Mediterranean invertebrates. Pure Appl Chem 58:375–386
7. Cimino G, Sodano G (1989) The chemical ecology of Mediterranean Opisthobranchs. Chem Scr 29:389–394
8. Garson M (1986) Biosynthesis of the novel diterpene isonitrile diisocyanoadociane by a marine sponge of the *Amphimedon* genus: incorporation studies with sodium cyanide-^{14}C and sodium acetate-2–^{14}C. J Chem Soc Chem Commun pp 35–36
9. Fookes CJR, Garson MJ, MacLeod JK, Skelton BW, White AH (1988) Biosynthesis of diisocyanoadociane, a novel diterpene from the marine sponge *Amphimedon* sp. crystal structure of a monoamide derivative. J Chem Soc, Perkin Trans 1003–1011
10. Karuso P, Scheuer PJ (1989) Biosynthesis of isocyanoterpenes in sponges. J Org Chem 54:2092–2095
11. Chang CWJ, Scheuer PJ (1990) Biosynthesis of marine isocyanoterpenoids in sponges. Comp Biochem Physiol 97B:227–233
12. Bayer FM (1961) The shallow-water octocorallia of the West Indian region. Martinus Nijoff, The Hague
13. Fenical W (1987) Marine soft corals of the genus *Pseudopterogorgia*: a resource for novel anti-inflammatory diterpenoids. J Nat Prod 50:1001–1008

14. Rodriguez AD (1995) The natural products chemistry of West Indian gorgonian octocorals. Tetrahedron 51:4571–4618
15. Look SA, Fenical W, Matsumoto GK, Clardy J (1986) The pseudopterosins: a new class of anti-inflammatory and analgesic diterpene pentosides from the marine sea whip *Pseudopterogorgia elisabethae* (Octocorallia). J Org Chem 51:5140–5145
16. Roussis V, Wu Z, Fenical W (1990) New anti-inflammatory pseudopterosins from the marine octocoral *Pseudopterogorgia elisabethae*. J Org Chem 55:4916–4922
17. Look SA, Fenical W (1987) The seco-pseudopterosins, new anti-inflammatory diterpene-glycosides from a Caribbean gorgonian octocoral of the genus *Pseudopterogorgia*. Tetrahedron 43:3363–3370
18. Look SA, Fenical W, Jacobs R, Clardy J (1986b) The pseudopterosins: anti-inflammatory and analgesic natural products from the sea whip *Pseudopterogorgia elisabethae*. Proc Natl Acad Sci USA 83:6238–6240
19. Ata A, Kerr RG, Moya C, Jacobs R (2003) Identification of anti-inflammatory diterpenes from the marine gorgonian *Pseudopterogorgia elisabethae*. Tetrahedron 59:4215–4222
20. Mayer AM, Jacobson PB, Fenical W, Jacobs RS, Glaser KB (1998) Pharmacological characterization of the pseudopterosins: novel anti-inflammatory natural products isolated from the Caribbean soft coral, *Pseudopterogorgia elisabethae*. Life Sci 62:PL401–PL407
21. Garson MJ (1993) The biosynthesis of marine natural products. Chem Rev 93:1699–1733
22. Kohl AC, Kerr RG (2003) A postulated biosynthetic origin of pseudopterosins and a proposed chemoenzymatic production method. J Ind Microbiol 30:495–499
23. Coleman AC, Mydlarz LD, Kerr RG (1999) In vivo and in vitro investigations into the biosynthetic relatedness of the pseudopterosins. Org Lett 1:2173–2175
24. Gershenzon J, Croteau RB (1993) Terpenoid biosynthesis: the basic pathway and formation of monoterpenes, sesquiterpenes, and diterpenes. Lipid Metab Plants 339–388
25. Davis EM, Croteau R (2000) Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpenes. Biosynthesis: aromatic polyketides, isoprenoids, alkaloids. Top Curr Chem 209:53–95
26. Wildung MR, Croteau RB (1996) A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of taxol biosynthesis. J Biol Chem 271:9201–9204
27. Vogel BS, Wildung MR, Vogel G, Croteau RB (1996) Abietadiene synthase from Grand Fir (*Abies grandis*). J Biol Chem 271:23262–23268
28. Back K, Chappell J (1996) Identifying functional domains within terpene cyclases using a domain-swapping strategy. Proc Natl Acad Sci USA 93:6841–6845
29. Starks CM, Back K, Chappell J, Noel JP (1997) Structural basis for cyclic terpene biosynthesis by tobacco 5-epi-aristolochene synthase. Science 277:1815–1820
30. Bohlmann J, Crock J, Jetter R, Croteau R (1998) Terpenoid-based defenses in conifers: cDNA cloning, characterization, and functional expression of wound-inducible (E)-α-bisabolene from grand fir (*Abies grandis*). Proc Natl Acad Sci USA 95:6756–6761
31. Rynkiewicz M, Cane D, Christianson D (2001) Structure of trichodiene synthase from *Fusarium sporotrichioides* provides mechanistic inferences on the terpene cyclization cascade. Proc Natl Acad Sci USA 98:13543–13548
32. Coleman A, Kerr RG (2000) Radioactivity-guided isolation and characterization of the bicyclic pseudopterosin diterpene cyclase product from *Pseudopterogorgia elisabethae*. Tetrahedron 56:9569–9574
33. Rodriguez AD, Ramirez C (2001) Serrulatane diterpenes with antimycobacterial activity isolated from the West Indian sea whip *Pseudopterogorgia elisabethae*. J Nat Prod 64:100–102
34. Kohl AC, Kerr RG (2003) Pseudopterosin biosynthesis: aromatization of the diterpene cyclase product, elisabethatriene. Mar Drugs 1:54–65
35. Ferns T, Kerr RG (2005) Oxidations of erogorgiaene in pseudopterosin biosynthesis. Tetrahedron 61:12358–12365
36. Ferns T, Kerr RG (2005) Identification of amphilectosins as key intermediates in pseudopterosin biosynthesis. J Org Chem 70:6152–6157