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Bipinnapterolide B, a bioactive oxapolycyclic diterpene from the Colombian gorgonian coral *Pseudopterogorgia bipinnata*

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Abstract—A unique oxapolycyclic diterpenoid, named bipinnapterolide B, has been isolated from the Colombian gorgonian octocoral *Pseudopterogorgia bipinnata*, and its structure was deduced from spectral and X-ray diffraction studies. Bipinnapterolide B (**3**) inhibits the growth of *Mycobacterium tuberculosis*.
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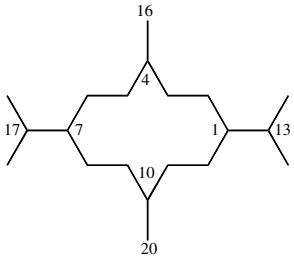
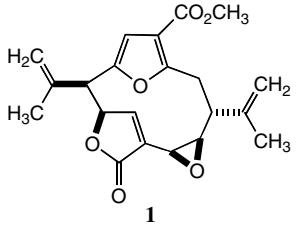
Starting in the early 1980s, chemical studies of Caribbean gorgonian corals of the genus *Pseudopterogorgia* have revealed that the majority of these gorgonian species produce diterpenoids of unusual structure types.[1] One such family of *Pseudopterogorgia* metabolites is the pseudopterane diterpenoids.[2] Several Caribbean species of *Pseudopterogorgia* (*P. acerosa*, *P. kallos*, and *P. bipinnata*) are well-known for their ability to biosynthesize diterpenes based on the highly symmetrical pseudopterane skeleton.[3] This class of metabolites, exemplified by pseudopterolide (**1**) and kallolide A (**2**), is of great interest to synthetic and natural products chemists because of their structural complexity and their desirable pharmacological value.[4] As part of an ongoing effort to discover new antituberculosis agents from marine sources, we report here the isolation, structure determination, and biological evaluation of bipinnapterolide B (**3**), a previously unknown metabolite of the pseudopterane family of diterpenes from extracts of a Colombian specimen of *P. bipinnata*.[5]

Partially air-dried specimens of *P. bipinnata* (Verill, 1864) collected from Old Providence Island, Colombia (13°21′N 81°22′W) in March 15, 2002, were frozen, freeze-dried (0.11 kg), blended with 1:1 MeOH/$CHCl_3$ (10×1 L), and filtered to yield a brown residue (25.0 g) that was suspended in H_2O and extracted with hexane, $CHCl_3$, and EtOAc. Chemical analysis of the bioactive hexane extract (12.0 g) led to the isolation of known compounds kallolide A acetate (27 mg, 0.11%), gersemolide (1.0 mg, 0.004%), and pinnatin B (5 mg, 0.02%), whereas the analysis of its also bioactive $CHCl_3$ extract afforded kallolide C (2.0 mg, 0.008%), kallolide C acetate (14.3 mg, 0.06%), bipinnapterolide A (37 mg, 0.15%), kallolide D (2.5 mg, 0.01%), kallolide E (4.0 mg, 0.02%), kallolide F (3.0 mg, 0.01%), and kallolide G (2.0 mg, 0.01%).[6] Having as target the investigation of the extensive chemodiversity of *P. bipinnata* and the evaluation of its secondary metabolites as potential



pseudopterane skeleton



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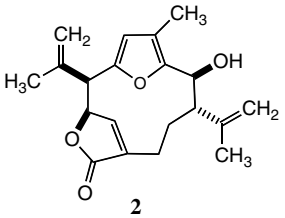

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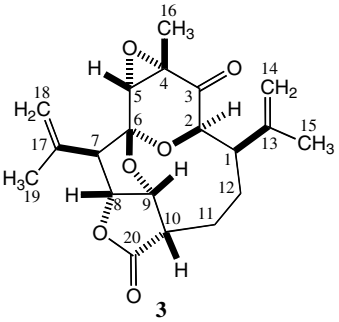

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Keywords: *Pseudopterogorgia bipinnata*; Tuberculosis; Caribbean gorgonian octocorals; *Mycobacterium tuberculosis*; Diterpenes.
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antitubercular agents, we studied a small fraction from the hexane extract that had not been previously investigated. Thus, bipinnapterolide B (**3**, 3.0 mg, 0.01%) was isolated and purified after consecutive size exclusion (Bio-Beads SX-3 in toluene) and Si gel column chromatography with mixtures of hexane/EtOAc, followed by normal-phase HPLC (Ultrasphere Si gel) with 10% 2-propanol in hexane.

Bipinnapterolide B (**3**) was isolated as white crystals, $[\alpha]_D^{20}$ −2.0° (*c* 1.0, $CHCl_3$), and the formula $C_{20}H_{24}O_6$ was established by HRESIMS signifying nine sites of unsaturation.[7] IR spectroscopy indicated the presence of olefin (3088 and 1644 cm^{-1}) and epoxy (strong ring deformation bands at 905 and 873 cm^{-1}) functionalities, in addition to γ-lactone (1763 cm^{-1}) and ketone (1716 cm^{-1}) carbonyl groups. Because the UV spectrum of **3** showed only end absorption, the carbonyl groups were non-conjugated. Interestingly, compound **3** gave rise to ^1H NMR spectra characterized by an abundance of one-proton and three-proton singlet signals, suggesting the presence of many isolated proton-spin systems. The ^1H and ^{13}C NMR (Table 1) identified four oxygen-bearing methines [δ_C 85.7, 82.2, 81.6, 61.1; δ_H 5.07 (d, 1H, $J = 5.7$ Hz), 5.19 (dd, 1H, $J = 5.7$, 8.1 Hz), 4.66 (br d, 1H, $J = 1.6$ Hz), 3.38 (s, 1H)]; two carbonyl groups, one ester (δ 177.4) and one ketone (δ 203.6); two oxygen-bearing sp^3 quaternary carbons (δ 105.9 and 60.3); four vinyl carbons, of which two were quaternary (δ 146.3 and 140.4) and two were terminal (δ 115.7 and 111.3); two sp^3 methylenes (δ 26.4 and 25.5); three sp^3 methines (δ 59.4, 47.3, and 42.5); and three methyl groups all of which were attached to quaternary carbons (δ 23.4, 22.9, and 14.4). Spectral evidence thus demanded that compound **3** was pentacyclic with two olefins and two carbonyl groups. These NMR signals, coupled with the IR spectrum, indicated that bipinnapterolide B lacked the typical α,α′,β-trisubstituted furan and α,γ-disubstituted α,β-unsaturated γ-lactone constellations found in **1** and **2**.[2,4] On the other hand, the olefin absorptions at 3088 and 1644 cm^{-1}, the broad singlets at δ 5.13 (1H), 4.83 (two overlapped signals), and 4.70 (1H), along with the three-proton singlets at δ 1.92 and 1.79 in the ^1H NMR spectrum and carbon resonances at δ 146.3 (C), 140.4 (C), 115.7 (CH_2), and 111.3 (CH_2) in the ^{13}C NMR spectrum, were ascribed to the same two isopropenyl groups found in **1** and **2**. The ^{13}C NMR lines observed at δ 61.1 (CH), 60.3 (C), and 14.4 (CH_3), combined with the one-proton signal at δ 3.38 and three-proton singlet at δ 1.48, were confidently assigned to an isolated methyl-bearing trisubstituted epoxide.[8]

After association of all carbon signals with the corresponding signals for directly bonded protons via an HMQC experiment in $CDCl_3$, ^1H–^1H COSY and HMBC spectral measurements were recorded (Table 1). The COSY spectrum allowed a continuous chain of ^1H–^1H coupling from H-8 to H_2-12 to be discerned and also revealed further coupling between H_2-12 and H-1 and from H-1 to H-2. Interestingly, there was essentially no coupling between H-7 and H-8 thus indicating that the dihedral angle between these protons approached 90°. The remainder of the ^1H–^1H COSY spectrum of **3** revealed no further vicinal couplings.

In the HMBC experiment, the H_3-19 protons showed heteronuclear couplings to C-7 (δ 59.4), C-17 (δ 140.4), and C-18 (δ 115.7), and the H-7 signal correlated to C-6 (δ 105.9), C-8 (δ 85.7), and C-9 (δ 82.2), in

Table 1. ^1H NMR (400 MHz), ^{13}C NMR (75 MHz), ^1H–^1H COSY, NOESY, and HMBC spectral data for bipinnapterolide B (**3**)[a]

Atom	δ_H, mult, intrgt (*J* in Hz)	δ_C, mult[b]	^1H–^1H COSY	NOESY	HMBC[c]
1	2.51, br m, 1H	47.3 (CH)	H-2, H-12αβ	H-2, H-14α	H-2, H-14αβ, H_3-15
2	4.66, br d, 1H (1.6)	81.6 (CH)	H-1	H-1, H_3-15	H-1
3		203.6 (C)			H-2, H_3-16
4		60.3 (C)			H-5, H_3-16
5	3.38, s, 1H	61.1 (CH)		H_3-16, H_3-19	H_3-16
6		105.9 (C)			H-2, H-5, H-7, H-8, H-9
7	3.27, s, 1H	59.4 (CH)		H-8, H-18β, H_3-19	H-18αβ, H_3-19
8	5.07, d, 1H (5.7)	85.7 (CH)	H-9	H-7, H-10, H-18α, H_3-19	H-7
9	5.19, dd, 1H (5.7, 8.1)	82.2 (CH)	H-8, H-10	H-10, H_3-19	H-7, H-8
10	2.69, ddd, 1H (2.5, 6.5, 8.1)	42.5 (CH)	H-9, H-11αβ	H-8, H-9	
11αβ	2.34, br m, 1H; 2.11, br m, 1H	25.5 (CH_2)	H-10, H-12αβ		H-10
12αβ	2.11, br m, 1H; 1.89, m, 1H	26.4 (CH_2)	H-1, H-11αβ		H-1, H-2, H-10
13		146.3 (C)			H-1, H-12αβ, H_3-15
14α	4.70, br s, 1H	111.3 (CH_2)	H-14β, H_3-15	H-14β, H_3-15	H_3-15
14β	4.83, br s, 1H		H-14α, H_3-15	H-1, H-14α	
15	1.79, s, 3H	22.9 (CH_3)	H-14αβ	H-2, H-14β	H-14αβ
16	1.48, s, 3H	14.4 (CH_3)		H-5	H-5
17		140.4 (C)			H-7, H-8, H_3-19
18α	5.13, br s, 1H	115.7 (CH_2)	H-18β, H_3-19	H-18β, H_3-19	H-7, H_3-19
18β	4.83, br s, 1H		H-18α, H_3-19	H-7, H-18α	
19	1.92, s, 3H	23.4 (CH_3)	H-18αβ	H-5, H-7, H-8, H-9, H-18α	H-7, H-18αβ
20		177.4 (C)			H-8, H-9

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in parts per million relative to TMS.
[b] ^{13}C NMR multiplicities were obtained from a DEPT-135 experiment.
[c] Protons correlated to carbon resonances in the ^{13}C column.

addition to C-17 and C-18. In a similar fashion, H_3-15 was correlated to C-1 (δ 47.3), C-13 (δ 146.3), and C-14 (δ 111.3), and the H-1 signal correlated to C-13, C-2 (δ 81.6), and C-12 (δ 26.4). The chemical shifts of C-8 suggested an ester linkage at that point, and accordingly, the C-20 lactone carbonyl showed HMBC correlations to H-8. The observation of strong HMBC correlations from C-6 to H-5, H-7, and H-8 allowed us to establish confidently that the ketal carbon was flanked by C-5 and C-7. Furthermore, observation of three-bond proton-carbon connectivity in the HMBC experiment between carbon resonance C-6 and H-2 (δ 4.66) and H-9 (δ 5.19) allowed the attachment of the ketal oxygen atoms through C-2 and C-9. The long-range correlations between the lactone carbonyl carbon at δ 177.4 (C-20) and H-9 and between the ketone carbonyl at δ 203.6 (C-3) and H-2 further confirmed these connectivities. At this point, the link of the trisubstituted epoxy moiety through C-3 and C-6 (deduced from strong HMBC cross-peaks between H_3-16 and C-3 and H-5 and C-6) allowed the complete planar structure for **3** to be assigned. Confirmation of the substitution pattern of the adjoining γ-lactone, oxolane, 3-pyranone, and oxirane ring systems in **3**, as well as the connectivities of all these rings came from additional HMBC and NOESY correlations found in Table 1. Applying these combined NMR methods resulted in the unambiguous assignment of all the protons and carbons as listed in Table 1.

The relative stereochemistries for all of the substituents about the complex pentacyclic array in **3** were determined by analysis of proton-proton coupling constants and NOE experiments obtained in $CDCl_3$ (Table 1). The methine protons H-1 and H-2 exhibited a *J* value of 1.6 Hz, establishing a cis-relationship of H-1 and H-2. This assignment was further supported by the strong NOESY correlations of H-1 (α-orientation in planar conformation) and H-2. Furthermore, NOEs between H-9 and methine protons H-8 and H-10, and NOE

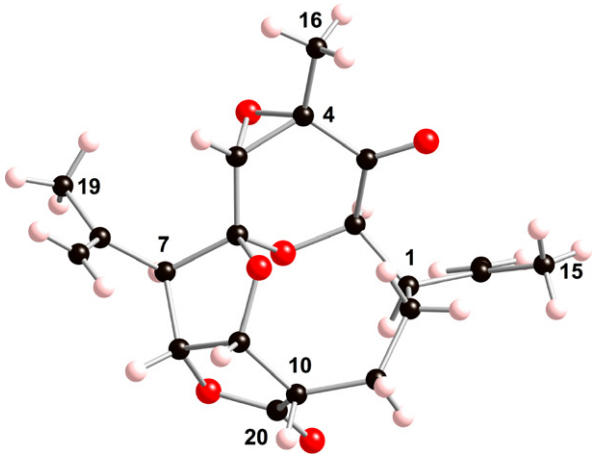
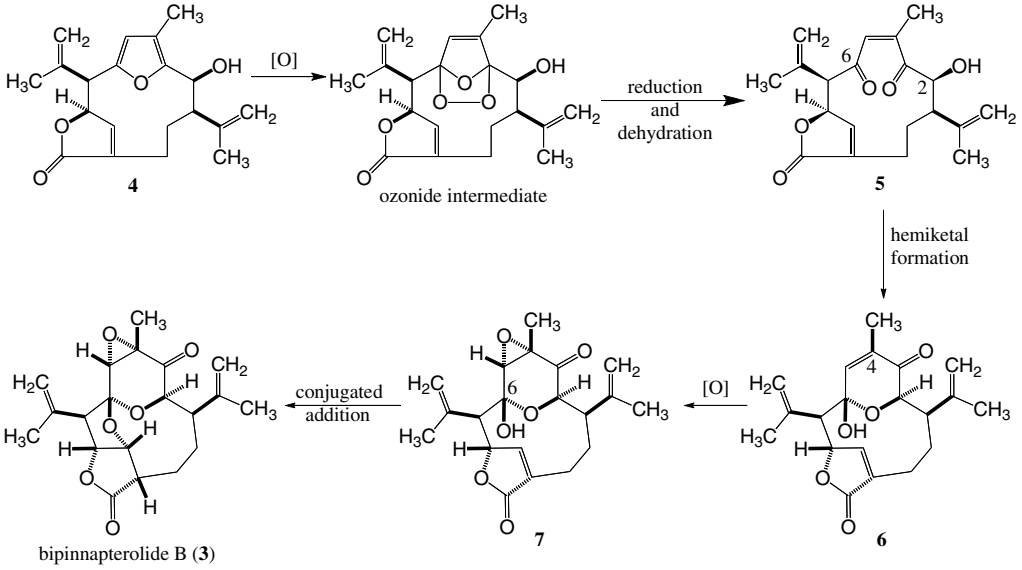


Figure 1. Illustration of the X-ray crystal structure of **3**.

cross-peaks between H-8 and H_3-19, H_3-19 and H-5, and H-5 and H_3-16, placed all of these protons close to each other on the β face. Regrettably, the C-1,2 constellation could not be correlated with the C-4 through C-10 array due to the absence of NOEs between them. Fortunately, compound **3** crystallized, and an X-ray analysis provided the structure along with the complete relative stereochemistry (see Fig. 1). Interestingly, bipinnapterolide B crystallized in the space group P1 with two molecules per unit cell.[9] Thus, the overall relative stereochemistry for bipinnapterolide B (**3**) was assigned as 1*R**,2*R**,4*S**,5*R**,6*R**,7*R**,8*R**,9*R**,10*S**.

The isolation of several furanopseudopterane diterpenoids and **3** from the same specimen of *P. bipinnata* provides circumstantial support that bipinnapterolide B (**3**) might be synthesized in vivo by subsequent oxidation of the furan ring of an as yet to be discovered C-1 epimer of kallolide A (**2**) (i.e., compound **4**) to afford 1,4-enedione intermediate **5**. The latter compound could then undergo intramolecular ketal formation to yield



Scheme 1. Proposed pathway for bipinnapterolide B (**3**) biosynthesis.

3(2*H*)-pyranone **6**. Further oxidation at Δ^4 might lead to intermediate **7**, which in turn, could undergo intramolecular conjugated addition of the C-6 hydroxyl to the α,β-unsaturated-γ-lactone leading to bipinnapterolide B (**3**) (Scheme 1).

In our primary in vitro antituberculosis screen against *Mycobacterium tuberculosis* $H_{37}Rv$ at 128 μg/mL, bipinnapterolide B (**3**) caused 66% inhibition. Further study on the antitubercular properties of **3** is underway.

Bipinnapterolide B (**3**) is an exceptional metabolite in various respects. In addition to its promising biomedical potential, it is the combination of two oxabridges across the 12-membered pseudopterane carbon skeleton (between C-2/C-6 and C-6/C-9) leading to a complex oxapolycyclic array, plus the observation that the C-1 isopropylene group is pointing upward (β configuration) that makes this natural product quite unique. Moreover, the latter structural feature suggests that **3** must ultimately originate from a cembrane-type pecursor that belongs to the β series and that is prone to undergo a two-carbon ring contraction process leading to a pseudopterane intermediate such as **4**.[10]

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