Highly Oxygenated Pseudopterane and Cembranolide Diterpenes from the Caribbean Sea Feather *Pseudopterogorgia bipinnata*

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A chemical study of the sea feather *Pseudopterogorgia bipinnata* from Colombia has produced four known metabolites, namely, kallolide A (**1**), bipinnatin A (**2**), bipinnatin C (**3**), and bipinnatin J (**4**), in addition to nine previously undescribed diterpenes possessing an uncommonly high level of oxygenation. One of them, bipinnapterolide A (**5**), is a new representative of the pseudopterane family of diterpenes possessing the rare 2,3-epoxy-1,4-dione moiety. The other metabolites, bipinnatins G−I (**6**−**8**) and bipinnatolides F−J (**9**−**13**), are highly oxygenated cembranolide diterpenes. Their chemical structures including relative stereochemistry were established by detailed analysis of the spectral data in addition to X-ray diffraction analysis and NMR spectral comparisons with known pseudopterane and cembranolide models.

Caribbean gorgonian octocorals of the genus *Pseudopterogorgia* are abundant and chemically rich marine invertebrates responsible for the production of several classes of metabolites many of which are of great interest because of their structural complexity and their desirable pharmacological value.[1,2] The studies of the natural products chemistry of these marine animals began in the late 1960s and emphasized the isolation of steroids and sesquiterpenoids. More recent studies of *Pseudopterogorgia* have revealed that the majority of these gorgonian species produce diterpenoids of unusual structure types.[2] Two such families of *Pseudopterogorgia* metabolites are the pseudopterane and the cembrane diterpenoids. Several Caribbean species of *Pseudopterogorgia* (*P. acerosa*, *P. kallos*, and *P. bipinnata*) are well-known for their ability to biosynthesize diterpenoids based on the 12-membered carbocyclic pseudopterane skeleton.[3] While the pseudopteranes appear to be taxonomically restricted to these *Pseudopterogorgia* (Gorgonacea) species and one species of *Gersemia* (Alcyonacea),[4] the cembranoids, on the other hand, are often found in many gorgonian and soft coral species.[5] Diterpenoids of the cembrane class are common metabolites of several West Indian gorgonian genera, especially *Eunicea*, *Plexaura*, and *Leptogorgia*.[2] On the other hand, *Pseudopterogorgia* species that produce cembranoid diterpenes do so in a much more restricted manner. Thus far, cembrane-based diterpenoids have been found in only two of the more than 15 species identified (*P. acerosa*[3c,k] and *P. bipinnata*[1,3j,6]). Of these, the major producer of cembrane derivatives is *Pseudopterogorgia bipinnata* Verrill (family Gorgoniidae), a widely distributed member of this genus. Since these gorgonian species contain both pseudopterane and cembrane diterpenes it appears that metabolites belonging to these skeleton classes are related biogenetically, a theory recently demonstrated in this laboratory.[3j] In a 1987 paper Fenical indicated that more than 15 cembrane derivatives were isolated from *P. bipinnata* collected in the Bahamas, but in fact only two cembrane-type structures, undemonstrated and without physical or chemical data, were disclosed.[1] In 1989, Wright et al. reported the isolation and structure elucidation of four cytotoxic furanocembranolides, denoted as bipinnatins A−D, from a specimen of *P. bipinnata* also collected in the Bahamas.[6] As part of our investigations of new antitumor and antituberculosis agents derived from marine sources, we report here the isolation and structure determination of nine previously undescribed pseudopterane and cembrane metabolites from extracts of a Colombian specimen of *P. bipinnata*. One of these, bipinnapterolide A (**5**), is structurally related to the known metabolite kallolide A (**1**),[7] and bipinnatins G−I (**6**−**8**) are highly oxygenated cembranolides which are structurally similar to the known metabolites bipinnatins A−D documented earlier by the Wright group.[6] In addition, we report the isolation from the same gorgonian specimen, of five new related cembranolides, **9**−**13**, which we have called bipinnatolides F−J, respectively.

A single collection of *P. bipinnata* from San Andrés Island, Colombia, produced 2.1 kg of the dry gorgonian coral. Partitioning of an aqueous suspension of the crude MeOH−$CHCl_3$ extract against hexane and H_2O gave lipophilic solubles (115.2 g) which accounted for 68.8% of the total organic content of *P. bipinnata*. Re-extraction of the lipid-free aqueous suspension with $CHCl_3$ followed by concentration yielded 43.3 g (25.8%) of organic solubles. The known metabolite kallolide A (**1**)[7] was isolated as a major constituent (3.76 g, yield = 2.24%, dry weight) as were the previously described bipinnatin A (**2**),[6] bipinnatin C (**3**),[6] and bipinnatin J (**4**).[3j] Bipinnapterolide A (**5**), bipinnatins G (**6**), H (**7**), and I (**8**), and bipinnatolides F (**9**), G (**10**), H (**11**), I (**12**), and J (**13**) were obtained pure from the latter extract after routine application of size-exclusion and Si gel column chromatography, and normal-phase high-pressure liquid chromatography (HPLC) (see Experimental Section). The molecular structures of diterpenes bipinnapterolide A (**5**) and bipinnatolide F (**9**), including relative stereochemistry, were confirmed by single-crystal X-ray crystallography. Two-dimensional NMR experiments of COSY, long-range COSY, and NOESY were widely used to established scalar and dipolar $^1H-^1H$ connectivities. $^1H-^{13}C$ correlations were obtained with HMQC and HMBC experiments. These efforts provided all the ^1H and ^{13}C NMR chemical shift and coupling data shown in Tables 1 and 2 for the new pseudopterane and cembranolide analogues **5**−**13** described here for the first time.[8]

Bipinnapterolide A (**5**) was isolated and purified by successive size exclusion (Bio-Beads SX-3 in toluene) and

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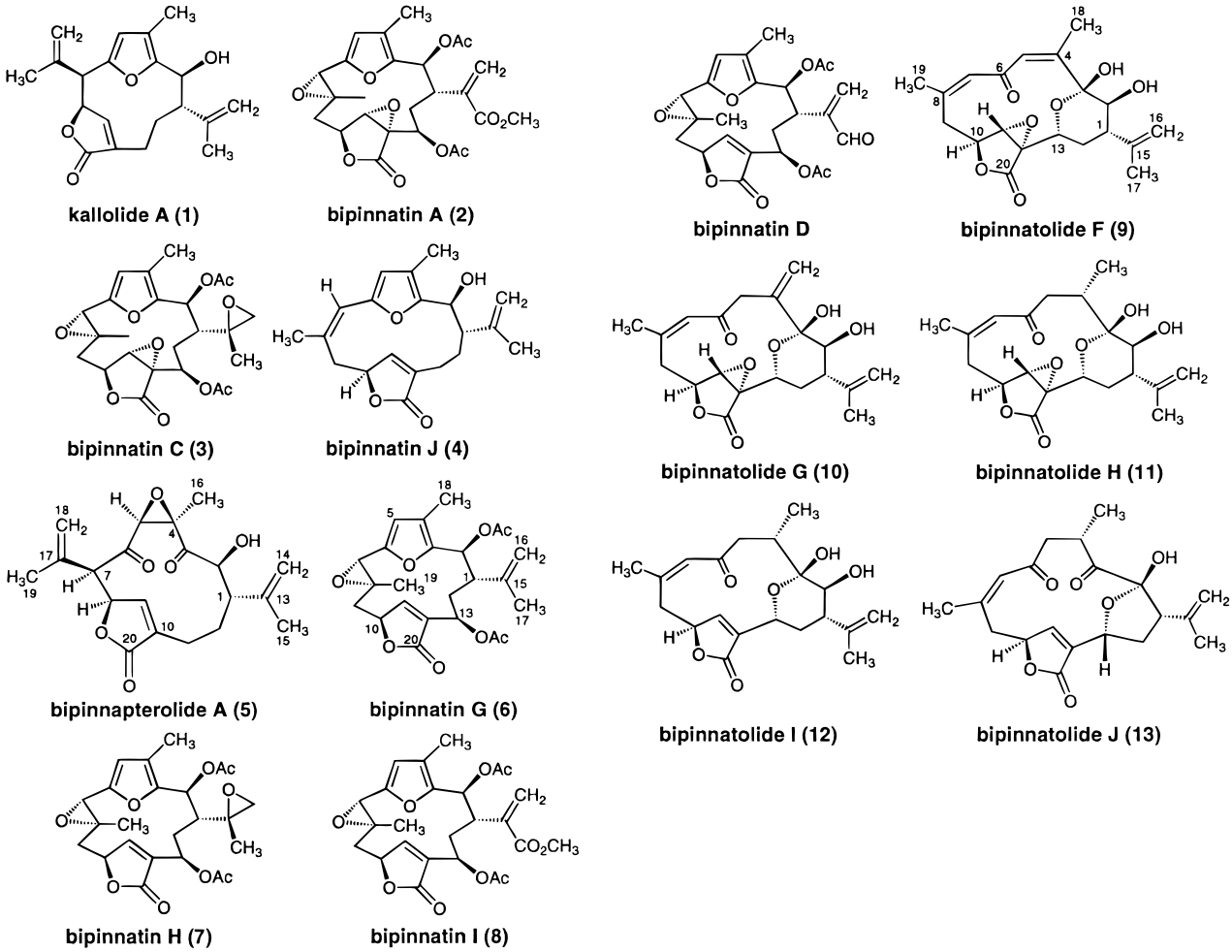
Chart 1



Si gel column chromatography with 3% acetone−$CHCl_3$. HRFABMS established its molecular formula as $C_{20}H_{24}O_6$, signifying nine sites of unsaturation. IR spectroscopy indicated the presence of hydroxyl (3551 cm^{-1}), olefin (3074, 1646 cm^{-1}), and epoxy (1264 cm^{-1}) functionalities, in addition to the α,β-unsaturated γ-lactone (1756 cm^{-1}) and ketone (1718, 1706 cm^{-1}) groups common to many of these metabolites. Interestingly, compound **5** gave rise to NMR spectra characterized by an abundance of broad signals of very low intensity, suggesting rapid intramolecular mobility near the NMR probe temperature. Notwithstanding, the ^1H and ^{13}C NMR (Table 1) identified three oxygen-bearing methines [δ_C 79.5 (d), 77.6 (d), 64.2 (d); δ_H 5.06 (1H, br s), 3.86 (1H, t, J = 11.0 Hz), 3.58 (1H, s)]; three carbonyl groups, one ester (δ 173.7), and two ketones (δ 208.5, 198.2); one oxygen-bearing quaternary carbon (δ 68.5); six vinyl carbons, of which three were quaternary (δ 141.4, 137.6, and 137.3), one was tertiary (δ 145.9), and two were terminal (both overlapped at δ 118.3); one allylic methylene (δ 20.4) and two allylic methines (δ 56.9 and 46.2); and three methyl groups all of which were attached to quaternary carbons (δ 21.9, 21.6, and 17.1). These NMR signals, coupled with the IR spectrum, indicated that bipinnapterolide A (**5**) lacked the α,α′-disubstituted β-methylfuran constellation found in kallolide A (**1**).[7] On the other hand, the carbonyl absorption at 1756 cm^{-1}, along with the one-proton signal at δ 6.64 in the ^1H NMR spectrum and carbon resonances at δ 173.7 (s), 145.9 (d), 137.6 (s), and 79.5 (d) in the ^{13}C NMR spectrum, were ascribed to the same α-substituted α,β-unsaturated γ-lactone functionality also found in kallolide A. The ^{13}C NMR lines observed at δ 141.4 (s), 137.3 (s), and 118.3 (two overlapped signals, t) and ^1H NMR peaks at δ 5.20 (2H, br s) and 5.03 (2H, br s) were confidently assigned to two isopropenyl groups. Consideration of the two ketones and epoxy groups in bipinnapterolide A (**5**), its UV spectrum (λ_{max} = 216 nm; ε 9800), and direct comparisons of the NMR spectral data for **5** with those of kallolide A (**1**), led to the conclusion that the α,α′-disubstituted β-methylfuran constellation found in kallolide A was transformed to a 2,3-epoxy-1,4-dione moiety in **5**. This conclusion was confirmed by the presence of two- and three-bond cross-peaks in the HMBC spectrum of **5** between the one-proton singlet at δ 3.58 (H-5) and the two carbonyl carbons at δ 208.5 and 198.2 ascribable to C-3 and C-6, respectively. The gross structure of **5** and all of the ^1H and ^{13}C chemical shifts associated with the molecule (see Table 1), which were assigned unambiguously by a series of 1D and 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC), suggested that bipinnapterolide A (**5**) is the 4,5-epoxy-3,6-dione analogue of kallolide A (**1**).

The relative stereochemistry of **5** was deduced from several 2D-NOE measurements. In the NOESY spectrum, the δ 5.06 proton (H-8) gave a cross-peak with the methine proton at δ 3.61 (H-7), which in turn gave a cross-peak with the epoxy singlet located at δ 3.58 (H-5) and the olefinic proton singlet at δ 6.64 (H-9). These observations established the relative stereochemistry at C-5, C-7, and C-8 shown in **5**. The stereochemistry at the C-4,5 epoxide, with H-5 cis to Me-16, was established by the strong cross-peak observed between these protons in the NOESY spectrum. Despite their strong vicinal coupling ($^3J_{1,2}$ = 11.0 Hz), no cross-peak was observed between H-1 and H-2 in the

Table 1. ^{1}H NMR and ^{13}C NMR Spectral Data for Bipinnapterolide A and Bipinnatins G−I[a,b]

	bipinnapterolide A (**5**)		bipinnatin G (**6**)		bipinnatin H (**7**)		bipinnatin I (**8**)	
position	δ, mult (J in Hz)	^{13}C, mult	δ, mult (J in Hz)	^{13}C, mult	δ, mult (J in Hz)	^{13}C, mult	δ, mult (J in Hz)	^{13}C, mult
1	1.90, m	46.2, d	4.29, t (10.8)	42.4, d	3.99, dd (10.2, 11.7)	39.4, d	4.65, t (10.5)	41.6, d
2	3.86, t (11.0)	77.6, d	5.75, d (11.6)	67.9, d	5.71, d (11.7)	68.1, d	6.14, d (11.4)	67.0, d
3		208.5, s		146.5, s		146.2, s		146.1, s
4		68.5, s		121.2, s		121.3, s		121.7, s
5	3.58, s	64.2, d	5.87, s	108.9, d	5.85, br s	109.0, d	5.87, br s	108.9, d
6		198.2, s		148.8, s		149.0, s		148.9, s
7	3.61, d (2.1)	56.9, d	4.12, br s	55.7, d	4.09, br s	55.6, d	4.11, br s	55.7, d
8	5.06, br s	79.5, d		57.0, s		57.0, s		56.9, s
9	6.64, br s	145.9, d	2.17, dd (3.0, 14.9)	39.9, t	2.16, dd (3.0, 15.0)	39.9, t	2.16, dd (3.0, 15.0)	40.0, t
9′			2.53, dd (4.1, 14.9)		2.52, dd (4.2, 15.0)		2.52, dd (4.2, 15.0)	
10		137.6, s	5.21, ddd (1.5, 3.0, 4.1)	77.7, d	5.12, ddd (1.5, 3.0, 4.2)	77.8, d	5.20, ddd (1.5, 3.0, 4.2)	77.8, d
11	2.13, m; 2.33, m	20.4, t	7.23, d (1.5)	151.1, d	7.25, d (1.5)	151.4, d	7.25, d (1.5)	151.4, d
12	1.69, m; 1.91, m	26.3, t		134.4, s		134.4, s		134.3, s
13		141.4, s	5.77, br d (5.8)	68.7, d	5.86, br d (6.3)	67.5, d	5.70, d (6.0)	69.0, d
14	5.03, br s	118.3, t	0.93, br d (15.5)	33.9, t	0.69, br d (15.0)	30.3, t	2.66, ddd (6.0, 10.0, 14.5)	33.1, t
14′	5.03, br s		2.37, ddd (5.8, 10.8, 15.5)		1.96, ddd (6.3, 10.2, 15.0)		0.99, br d (14.5)	
15	1.70, br s	17.1, q		146.5, s		56.9, s		140.5, s
16	1.63, br s	21.6, q	4.96, br s	113.6, t	2.52, d (4.5)	50.5, t	6.09, br d (1.5)	129.9, t
16′			5.05, br s		2.77, d (4.5)		6.33, br d (1.5)	
17		137.3, s	1.88, br s	21.3, q	1.58, br s	23.0, q		166.6, s
18	5.20, br s	118.3, t	1.99, br s	9.7, q	1.96, br s	9.7, q	2.00, br s	9.7, q
19	1.97, br s	21.9, q	0.96, br s	19.6, q	0.93, br s	19.6, q	0.96, br s	19.6, q
20		173.7, s		170.2, s		170.3, s		170.1, s
2-O*H*	2.60, br d (11.0)							
2-OCO*CH*$_3$			2.00, s	20.5, q	2.07, s	21.1, q	1.92, s	20.8, q
2-O*C*OCH$_3$				170.4, s		170.1, s		169.8, s
13-OCO*CH*$_3$			1.98, s	20.9, q	2.11, s	21.2, q	1.93, s	20.5, q
13-O*C*OCH$_3$				170.1, s		170.3, s		170.2, s
17-O*CH*$_3$							3.77, s	51.6, q

[a] Assignments were aided by $^{1}H-^{1}H$ COSY, spin splitting patterns, analysis of J values, HMBC and HMQC experiments, numbers of attached protons as measured from DEPT spectra, and chemical shift values. The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. [b] Data recorded in $CDCl_3$ solution at 300 MHz.

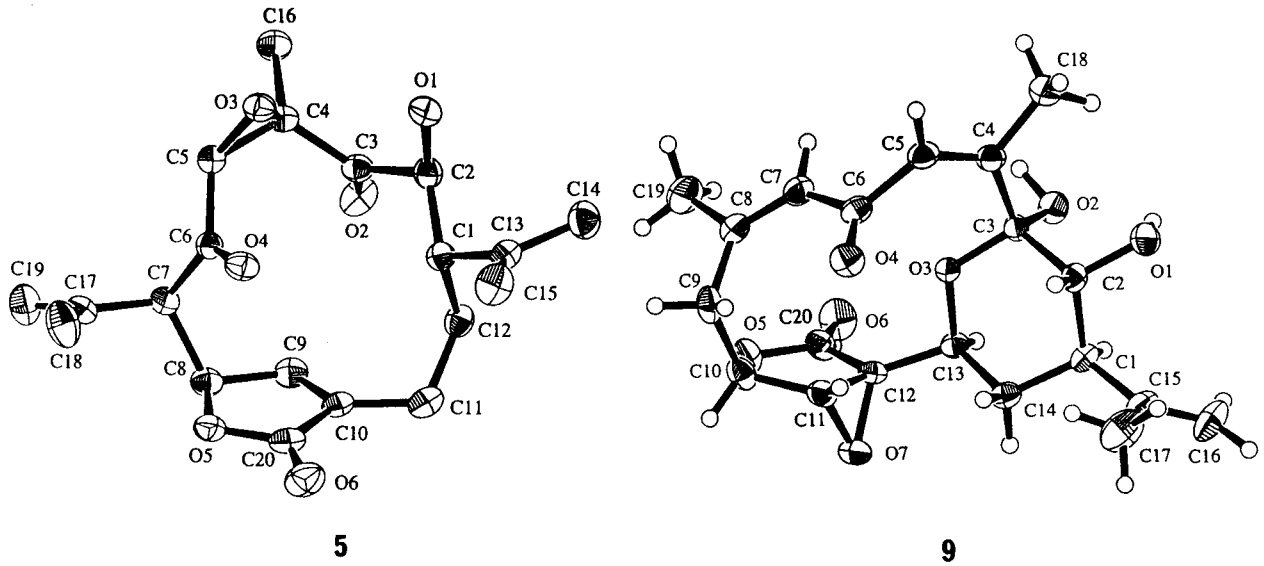


Figure 1. Computer-generated perspective drawings of the final X-ray crystallographic models of bipinnapterolide A (**5**) and bipinnatolide F (**9**). The thermal ellipsoids are drawn at the 40% probability level, and no information could be interpreted to assign the absolute configurations at the asymmetric centers. In the drawing shown for bipinnapterolide A (**5**) the hydrogens atoms are omitted for clarity. The drawing shown for bipinnatolide F is the enantiomer of the structure drawn as **9**.

NOESY spectrum of **5**. Thus, the determination of the β-orientation of H-1 in the carbocyclic ring is consistent with the proposed stereochemistry on the basis of its lack of NOE with H-2. Regrettably, the C-1,2 constellation could not be correlated with the C-4,5,7,8 array due to the absence of NOEs between them. Fortunately, compound **5** crystallized, and an X-ray analysis provided the structure with relative stereochemistry (see Figure 1).

Bipinnatin G (**6**), also isolated as a minor constituent, shared many spectral features in common with the known compound bipinnatin D.[6] High-resolution mass spectrometry established a molecular formula of $C_{24}H_{28}O_8$ for this compound, one oxygen atom less and two hydrogen atoms more than in the molecular formula of bipinnatin D. Except for the absence of an aldehyde proton and the corresponding carbonyl resonance [near δ 193.9 (d)],[6] the NMR spectra of **6** indicated that this compound contained many of the same functional groups as bipinnatin D, ie., olefin, α,β-unsaturated γ-lactone, epoxy, acetate, and β-methylfuran moieties. The major difference, however, was the replacement at C-1 of the α,β-unsaturated aldehyde with an isopropenyl group as evidenced by an intense three-proton singlet signal near 1.88 ppm [δ_C 21.3 (q)] indicative of a vinyl methyl functionality. Because there were otherwise no significantly large differences in both ^{1}H and ^{13}C chemical shift values at most positions in **6** when compared to bipinnatin D, it was quickly thought that bipinnatin G had structure **6**. Bipinnatin G has the same relative stereochemistry as that reported for bipinnatin D based upon 2D-NOE experiments[9] and comparison of their respective NMR chemical shifts and ^{1}H$-^1$H coupling constants.

The structural characterization of bipinnatin H (**7**) was carried out in an analogous manner. This metabolite was isolated as colorless crystals, $[\alpha]^{24}_D$ $-8.8°$, and the formula $C_{24}H_{28}O_9$, established by HREIMS, contained one oxygen atom more than in the molecular formula of bipinnatin G (**6**). Like **6**, compound **7** showed IR absorptions (3083, 1757, 1736, and 1236 cm^{-1}) that indicated the presence of olefin, α,β-unsaturated γ-lactone, acetate, and epoxy functionalities. That several of the same structural features found in **6** were also present in bipinnatin H (**7**) was supported by a UV absorption at λ_{max} = 214 nm (ϵ 19 000). Comparison of the ^{1}H and ^{13}C NMR spectra of **6** (see Table 1) with those of **7** confirmed the structural similarity of these compounds and revealed the presence of a feature unique to **7**. The α-substituted α,β-unsaturated γ-lactone and the α,α'-disubstituted β-methylfuran functionalities were assumed to be intact in **7** on the basis of similar IR, UV, and NMR data (compare signals of related H's and C's in Table 1). Bipinnatin H (**7**), however, had one oxygen-bearing methylene as indicated by a ^{13}C signal at δ 50.5 (t) and ^{1}H signals at δ 2.77 (1H, d, J = 4.5 Hz) and 2.52 (1H, d, J = 4.5 Hz). Also, resonances at δ 56.9 (s) and 57.0 (s) in the ^{13}C NMR spectrum of **7** confirmed the presence in this compound of two epoxy-bearing quaternary carbons, a feature not found in **6**. Clearly bipinnatin H, like known bipinnatin C (**3**),[6] contained an additional epoxy group at C-15,16 rather than a terminal olefin as in metabolite **6**. This would accommodate the eleven degrees of unsaturation as well as the number of oxygen atoms required by the molecular formula of **7**. A 2D ^{1}H NMR homonuclear correlation experiment (COSY) allowed the assignment of all the signals in the ^{1}H NMR spectrum of **7**. Chemical shifts of the protonated carbons were assigned by 2D ^{1}H$-^{13}$C heterocorrelation experiments (HMQC and HMBC) (see Table 1). The NOEs observed for **7**, particularly those between H-1 and Me-17, H-2 and Me-18, and H-11 with H-10, H-13, and Me-19, as well as the lack of NOE between H-1 and the proton signals ascribed to H-2 and H-13, correlated with a Dreiding model representing the relative stereochemistry shown in the structure of **7**. This compound has also a logical structure from a biosynthetic viewpoint. Thus, bipinnatin G (**6**) could be envisioned as a precursor for bipinnatin H (or vice versa) via oxidation, in this case at C-15,16.

Bipinnatin I (**8**) is most closely related to bipinnatin A (**2**), a previously reported metabolite from *P. bipinnata*.[6] This major metabolite, obtained as a colorless gum, was shown by HREIMS to have a molecular formula of $C_{25}H_{28}O_{10}$. Resonances at δ 170.2 (s), 170.1 (s), 169.8 (s), and 166.6 (s) in the ^{13}C NMR of **8** could be assigned to the carbonyls of four esters, thereby accounting for eight of the 10 oxygen atoms. Eight olefinic carbon resonances in the ^{13}C NMR (Table 1) indicated four carbon$-$carbon double bonds. Subtracting the eight sites of unsaturation required

by the carbonyl and olefinic functionalities, from the total of twelve sites required by the molecular formula, indicated that bipinnatin I (**8**) was tetracyclic. The ^{1}H and ^{13}C NMR data for **8** showed a strong resemblance to those displayed by bipinnatin A (**2**). Fortunately, compound **2** was in hand for spectral comparisons since we isolated it in 2.45 × 10^{-3}% yield from this gorgonian specimen. Careful examination of the NMR (Table 1) and IR spectra of **8** indicated the presence of the same α,β-unsaturated methyl ester, α,α′-disubstituted β-methylfuran, trisubstituted epoxide, and secondary acetate substructures found in **2**, but also revealed that these spectra appeared not to have the signals for the α,β-epoxy γ-lactone present in **2**. Thus, the only difference was the replacement of the α,β-epoxy γ-lactone in **2** with an α,β-unsaturated γ-lactone in **8** as evidenced by the carbon NMR resonances observed at δ 170.1 (s), 151.4 (d), 134.3 (s), and 77.8 (d) and the proton resonances observed at δ 7.25 (d, 1H, J = 1.5 Hz, H-11) and 5.20 (ddd, 1H, J = 1.5, 3.0, 4.2 Hz, H-10). Assignment of the relative stereochemistries of **8** was based upon 2D-NOE experiments[10] and comparison of the NMR chemical shifts and ^{1}H−^{1}H coupling constants with those of compounds **2**, **6**, and **7**.

Bipinnatolide F (**9**) was isolated as a UV-active (λ_{max} = 204, 244 nm) crystalline substance that analyzed for $C_{20}H_{24}O_7$ by combined HREIMS and ^{13}C NMR methods. The IR spectrum was consistent with the presence of epoxide (1287 cm^{-1}), ester (1782 cm^{-1}) and conjugated enone (1670 cm^{-1}) carbonyl functionalities. Intense broad absorptions near 3575, 3564, and 3364 cm^{-1} also suggested several hydroxyl groups. A most striking detail observed in the ^{1}H NMR spectrum of **9** was the absence of the signals usually ascribed to the α,α′-disubstituted β-methylfuran functionality found in most bipinnatins.[6] In addition, the ^{1}H NMR spectrum in $CDCl_3$ showed signals for four protons attached to carbons bearing oxygen [δ 4.91 (1H, d, J = 8.1 Hz, H-10), δ 4.87 (1H, dd, J = 2.4, 12.2 Hz, H-13), δ 4.34 (1H, s, H-11), and δ 3.51 (1H, dd, J = 6.0, 10.5 Hz, H-2)] and three vinyl methyl signals: δ 1.98 (3H, br s, Me-18), δ 1.95 (3H, br s, Me-19), and δ 1.70 (3H, br s, Me-17). The ^{13}C NMR spectrum exhibited twenty signals separated by DEPT into seven quaternary carbons (C-3, C-4, C-6, C-8, C-12, C-15, and C-20), seven methines (C-1, C-2, C-5, C-7, C-10, C-11, and C-13), three methylenes (C-9, C-14, and C-16), and three methyl groups (Me-17, Me-18, and Me-19). Two of the signals were due, respectively, to a conjugated ketone and an ester carbonyl [δ 196.1 (s), 171.0 (s)], six were oxygenated carbons [δ 97.5 (s), 77.9 (d), 74.2 (d), 64.4 (d), 59.8 (d), 58.8 (s)], and six were olefinic [δ 148.9 (s), 144.1 (s), 141.8 (s), 131.0 (d), 128.3 (d), and 115.1 (t)]. The resonance at δ 97.5 (s) suggested a cyclic hemiacetal group, and those at δ 171.0 (s), 77.9 (d), 59.8 (d), and 58.8 (s) indicated a α,β-epoxy γ-lactone moiety.[6] Moreover, the ^{13}C resonances at δ 196.1 (s), 148.9 (s), 141.8 (s), 131.0 (d), and 128.3 (d) were ascribed to a tetrasubstituted cross-conjugated dienone functionality,[4,11] and from the ^{13}C NMR resonances at δ 144.1 (s), 115.1 (t), and 19.3 (q), we concluded that bipinnatolide F (**9**) also possessed an isopropylene group. Information gleaned from COSY, NOESY, HMQC, and HMBC spectra led to formulation of structure **9** for bipinnatolide F, and this was confirmed by X-ray crystallographic analysis.

A perspective ORTEP plot of the antipode of **9** is shown in Figure 1. The X-ray experiment did not define the absolute configuration; the enantiomer shown as structure **9** has been chosen arbitrarily to conform with the absolute configuration of kallolide A (**1**) at C-1.[12] This molecule contains an unprecedented 3,13-bridged cembrane carbocyclic skeleton which also features a rare cross-conjugated dienone functionality and a hydroxyl group at C-2 in the β configuration. The overall conformation of the tetracyclic cembrane skeleton in the present structure has severely restricted rotation. Observation of an NOE between H-1/H-13, H-1/H-14β, H-2/H-14α, H-2/Me-18, H-5/Me-18, H-7/Me-19, H-9α/H-10, H-9α/Me-19, H-9β/H-11, H-11/H-14α, and H-13/H-14β indicates that these proton pairs are on the same face of the molecule, and most importantly, the fact that no NOE was observed between H-1 and H-2 or between protons H-10 and H-11, confirmed that the solution conformation is similar to that in the solid state as elucidated by X-ray analysis. In such a conformation, only one of the two C=C groups of the dienone (Δ^7) is oriented so that there can be effective overlap with the π orbitals of the C=O group (see Figure 1). Curiously, the allylic proton H-9β is unusually deshielded to δ 4.11 indicating that it must lie along the transverse axis of the C=O bond of the dienone system in **9**. The geometry is such that H-9β, which lies close to the C=O bond, is in the deshielding portion of the induced magnetic field. Interestingly, while diterpenes **1** and **9** contain similarly functionalized chiral groups with identical relative stereochemistry (i.e., C-1, C-2, and the carbon atom bearing the γ-lactonic methine), the relative orientation of the C-1 isopropylene group in bipinnatolide F (**9**) is clearly the opposite to that found in the corresponding substructure of most cembrane-based diterpenes found in the Caribbean region.[2] Since the absolute configuration of kallolide A (**1**) has been recently established,[12] and its structure has been correlated chemically with that of furanocembranoid bipinnatin J (**4**),[3j] a plausible precursor to bipinnatolide F (**9**) upon hydrolysis of the furan ring and further oxidation on the α face of the molecule, one can safely assume that **9** also has the same absolute configuration at all common chiral centers.

Bipinnatolide G (**10**) was isolated as a UV-active (λ_{max} = 206, 242 nm) crystalline solid also with a molecular formula of $C_{20}H_{24}O_7$ established from HRFABMS data. Strong IR bands due to hydroxyl (3508, 3289 cm^{-1}), epoxide (1256 cm^{-1}), ester (1783 cm^{-1}) and conjugated ketone (1674 cm^{-1}) functionalities were clearly detected. The ^{1}H NMR spectrum in acetone-d_6 showed two pairs of methylene signals at δ 5.55 and 5.27 (each 1H, br s) and at δ 4.83 and 4.79 (each 1H, br s) indicative of two terminal olefins. Four signals near δ 4.92, 4.66, 4.25, and 3.49 were attributed to methine protons on carbon atoms bearing oxygen (H-10, H-13, H-11, and H-2, respectively). The ^{13}C NMR spectrum exhibited 20 signals (2 CH_3, 5 CH_2, 6 CH, and 7 C) whose chemical shift values and multiplicities hinted the presence of a α,β-epoxy γ-lactone [δ 171.3 (s), 78.9 (d), 59.8 (s), and 59.7 (d)], a cyclic hemiacetal [δ 99.1 (s)], a β-methyl α,β-unsaturated enone [δ 198.6 (s), 147.6 (s), 127.1 (d), and 30.1 (q)], two terminal olefins [δ 147.0 (s), 144.8 (s), 118.9 (t), and 112.5 (t)], and one secondary hydroxyl group [δ 70.7 (d)]. At this stage we realized that the ^{1}H and ^{13}C NMR spectra of compound **10** were reminiscent of those observed for bipinnatolide F (**9**). Nevertheless, some major differences between these compounds in the ^{13}C NMR spectra were observed: the signals ascribed to C-4, C-5, and Me-18 in **9** showed considerable differences in chemical shifts and had shifted from δ 141.8, 131.0, and 20.8 in bipinnatolide F (**9**) to δ 144.8, 51.7, and 118.9 in **10**, respectively. Double bond isomerization of **9** at Δ^4 to an exocyclic position (between C-4 and C-18) would account for these spectral differences in **10**. Also, the appearance of an AB double doublet in the ^{1}H NMR spectrum of **10** at δ 3.29 (1H, J =

15.3 Hz, H-5β) and δ 3.06 (1H, J = 15.3 Hz, H-5α) was consistent with this contention. A NOESY experiment showed that H-1 and H-13, H-2 and H-5α, H-2 and H-14α, H-7 and Me-19, H-9α and H-10, and H-11 and H-14α were within NOE proximity which confirmed the relative stereochemistry depicted in **10**. Moreover, the similar ^{13}C NMR chemical shift values shown by both isomers for C-1, C-2, C-3, C-10, C-11, C-12, and C-13 clearly suggested the same relative stereochemistry at these sites.

The HRFABMS and ^{13}C NMR data for bipinnatolide H (**11**) were consistent with a molecular formula of $C_{20}H_{26}O_7$ which contained two hydrogen atoms more than in the molecular formula of bipinnatolide G (**10**). Intense IR bands at 3485 and 3473 cm^{-1} suggested several hydroxyl groups and bands at 1783 and 1670 cm^{-1} were ascribed to ester and conjugated enone carbonyl groups. Comparison of the ^{1}H and ^{13}C NMR spectra of **11** with those of bipinnatolide G (**10**) revealed only minor differences which were consistent with the replacement of the C-18 terminal methylene group in **10** with a secondary methyl group in **11**. For instance, in **10**, the H-18 methylene protons resonate at δ 5.55 and 5.27 (each 1H, br s) whereas in **11** the protons ascribed to Me-18 appear at δ 1.11 (3H, d, J = 7.1 Hz). The presence in the NMR spectra of a new methine signal [δ 2.49 (1H, m); δ 43.2 (d)] along with key long-range ^{1}H−^{13}C correlations obtained from HMBC experiments indicated that bipinnatolide H indeed had structure **11**. The gross structure of **11** was further supported by the NOESY data. The relative stereochemistry of **11** containing eight chiral centers was deduced from combination of the NOESY data with the ^{1}H−^{1}H coupling constants as shown in Table 2. The angular hydrogen H-13 and H-1 of the tetrahydropyran ring were suggested to be cis axial since a characteristic NOESY correlation was observed for H-13/H-1 with no cross-peaks between H-1/H-2. The spatial relationships of the γ-lactone, epoxide, and tetrahydropyran rings were further confirmed from NOESY cross-peaks for the H-9α/H-10, H-9α/Me-19, H-11/H-14α, and H-2/H-14α pairs in **11**. As in the case of **9** and **10**, the near absence of coupling (J < 1.0 Hz) of the δ 4.28 absorption for H-11 was attributed to the combined electronegativity effects of vicinal *trans*-coplanar oxygen atoms on the coupling strength of the H-10 and H-11 protons. The dihedral angles between these protons diminish the coupling strength of each proton, reducing their mutual coupling to less than 1 Hz. The strong NOE responses between the Me-18 protons and H-5α, H-7, and most importantly, the hydroxymethine proton H-2 established the α orientation of the former group. The proposed relative stereochemistry was also strongly supported by the strong NOE between H-7 and Me-19 suggesting that these protons were cis.

A molecular formula of $C_{20}H_{26}O_6$ was established for bipinnatolide I (**12**) from HRFABMS ([M + Na]$^+$ m/z = 385.1626) and ^{1}H and ^{13}C NMR data. Detailed comparisons of the NMR data of isomers **11** and **12** quickly revealed a close structural relationship between them. For instance, the ^{1}H NMR spectrum in $CDCl_3$ showed a signal at δ 7.46 (1H, d, J = 1.3 Hz, H-11) suggesting the presence in **12** of a α,β-unsaturated γ-lactone functionality. Other relevant features in the ^{1}H NMR spectrum were: three low field signals [δ 5.36 (br d, J = 7.4 Hz, H-10), δ 4.61 (dd, J = 2.6, 11.6 Hz, H-13), δ 3.53 (dd, J = 1.3, 10.3 Hz, H-2)] ascribable to protons attached to carbons bearing oxygen, and three methyl groups [δ 1.90 (d, J = 1.3 Hz, Me-19), δ 1.10 (d, J = 6.7 Hz, Me-18), δ 1.72 (br s, Me-17)]. The ^{13}C NMR spectrum of **12** also exhibited twenty signals (3 CH_3, 4 CH_2, 7 CH, and 6 C) indicating conjugated enone and ester carbonyls [δ 200.3 (s, C-6) and 172.4 (s, C-20), respectively], four oxygenated carbons [δ 96.8 (s, C-3); δ 80.3 (d, C-10); δ 72.6 (d, C-2); δ 63.9 (d, C-13)], and six olefinic carbons [δ 115.0 (t, C-16), δ 129.8 (d, C-7), δ 134.2 (s, C-12), δ 143.6 (s, C-15), δ 147.1 (s, C-8), δ 154.9 (d, C-11)]. While the ^{13}C NMR spectrum of **12** differed somewhat from that of bipinnatolide H (**11**), these differences were limited to the signals from carbons C-11, C-12, and C-14. The remainder of the ^{13}C NMR spectrum of **12** could be matched with that of **11**, although some of the signals were slightly shifted. From these data, it became certain that **12** no longer had an epoxide ring between C-11 and C-12 but instead possessed a α,γ-disubstituted butenolide ring. Interestingly, the ^{13}C NMR signal for C-14 in **12** seemed quite far downfield relative to structures **9**−**11** (the chemical shifts of the H-14$\alpha\beta$ protons also differed slightly). This peculiarity suggested that in bipinnatolides F−H, each having an α-oriented epoxide ring between C-11 and C-12, the atoms at C-14 come into some shielding cone from the epoxy oxygen. Moreover, the ^{13}C NMR chemical shift of C-14 (δ 34.6) in **12** was almost the same as for bipinnatolide J (**13**), which has a similar substitution pattern about the butenolide functionality (vide infra). A combination of ^{1}H−^{1}H COSY and HMBC experiments established the position of all of the functional groups in **12** unambiguously.[13] The Z geometry of the Δ^7 double bond in **12** was supported by a strong NOE between H-7 and Me-19. A NOESY experiment also revealed that proton pairs H-1/H-13, H-2/H-14α, H-2/Me-18, H-5α/H-7, H-5α/Me-18, H-9α/H-10, and H-9β/H-11 were also within NOE proximity.

A molecular formula of $C_{20}H_{24}O_6$ was established for compound **13**, named bipinnatolide J, from HRFABMS plus ^{1}H and ^{13}C NMR data. The IR spectrum contained carbonyl bands at 1746, 1730 (sh), and 1677 cm^{-1} consistent with the presence of ester, ketone, and conjugated enone groups, respectively, in addition to a strong hydroxyl stretching band at 3403 cm^{-1}. The ^{13}C NMR spectrum exhibited 20 signals (3 CH_3, 4 CH_2, 6 CH, and 7 C) whose chemical shift values and multiplicities hinted at the presence of a cyclic hemiacetal [δ 107.3 (s, C-2)], two trisubstituted olefins [δ 127.0 (d, C-7) and 148.9 (s, C-8); δ 153.1 (d, C-11) and 129.3 (s, C-12)] and one terminal olefin [δ 112.3 (t, C-16) and 139.6 (s, C-15)], two oxygen bearing carbons [δ 79.3 (d, C-10) and δ 70.0 (d, C-13)], and three carbonyl groups ascribable to: an ester [δ 171.7 (s, C-20)], a ketone [δ 210.3 (s, C-3)], and a conjugated enone [δ 200.1 (s, C-6)] functionality. Shortly after its purification, we realized that the NMR spectra of compound **13** were remarkably reminiscent of those of bipinnatolide I (**12**). A side-by-side comparison between the ^{13}C NMR data of compound **13** and those of isomer bipinnatolide I (see Table 2) rapidly pinpointed their structural similarities. Nevertheless, some major differences between these compounds were observed in the ^{13}C NMR spectra: bipinnatolide J (**13**) displayed an extra carbonyl resonance at δ 210.3 which was not present in bipinnatolide I and, moreover, the signals ascribed to C-1, C-2, C-3, and C-13 showed considerable differences in chemical shifts and had shifted from δ 47.3 (d), 72.6 (d), 96.8 (s), and 63.9 (d) in bipinnatolide I (**12**) to δ 55.6 (d), 107.3 (s), 210.3 (s), and 70.0 (t) in **13**, respectively. Placement in bipinnatolide J of a carbonyl at C-3 and a hemiacetal bridge between C-2 and C-13, with the C-2 hydroxyl group in the β-orientation, would account for these spectral differences. Consideration of ^{1}H and ^{13}C NMR data as well as other spectral data allowed the complete structure of this metabolite to be assigned as **13**. The multiple NOE's detected during a PSNOESY experiment

Table 2. ^{1}H NMR and ^{13}C NMR Spectral Data for Bipinnatolides F, G, H, I, and J[a]

	bipinnatolide F[b] (**9**)		bipinnatolide G[c] (**10**)		bipinnatolide H[d] (**11**)		bipinnatolide I[d] (**12**)		bipinnatolide J[d] (**13**)	
position	δ, mult (J in Hz)	^{13}C	δ, mult (J in Hz)	^{13}C	δ, mult (J in Hz)	^{13}C	δ, mult (J in Hz)	^{13}C	δ, mult (J in Hz)	^{13}C
1	2.62, ddd (3.2, 10.5, 12.2)	45.8	2.69, ddd (3.3, 10.5, 12.6)	44.8	2.45, ddd (3.2, 10.3, 11.8)	47.0	2.52,ddd (3.3,10.3,11.9)	47.3	3.23, dd (4.2, 12.3)	55.6
2	3.51, dd (6.0, 10.5)	74.2	3.49, br d (10.5)	70.7	3.39, dd (2.6, 10.3)	70.9	3.53, dd (1.3, 10.3)	72.6		107.3
3		97.5		99.1		97.5		96.8		210.3
4		141.8		144.8	2.49, m	43.2	2.60, m	43.8	3.20, ddd (3.3, 5.1, 6.6)	36.5
5α	5.81, s	131.0	3.06, d (15.3)	51.7	2.73, dd (10.6, 13.9)	46.9	2.66, dd (6.0, 13.2)	46.8	2.84, dd (3.3, 18.3)	48.3
5β			3.29, d (15.3)		1.92, dd (2.8, 13.9)		1.72, dd (5.8, 13.2)		2.05, dd (5.1, 18.3)	
6		196.1		198.6		200.9		200.3		200.1
7	6.23, br s	128.3	6.23, br s	127.1	6.28, br s	129.7	6.18, br s	129.8	6.06, br s	127.0
8		148.9		147.6		149.0		147.1		148.9
9α	2.58, dd (8.1, 12.8)	33.4	2.52, dd (7.8, 13.2)	33.6	2.51, dd (7.5, 12.9)	33.0	2.58, dd (7.4, 12.2)	35.0	2.70, dd (8.4, 12.6)	34.2
9β	4.11, d (12.8)		4.11, d (13.2)		4.18, d (12.9)		3.90, br d (12.2)		3.94, d (12.6)	
10	4.91, d (8.1)	77.9	4.92, d (7.8)	78.9	4.84, d (7.4)	78.3	5.36, br d (7.4)	80.3	5.38, d (8.4)	79.3
11	4.34, s	59.8	4.25, br s	59.7	4.28, s	60.4	7.46, d (1.3)	154.9	7.46, br d (0.9)	153.1
12		58.8		59.8		60.1		134.2		129.3
13	4.87, dd (2.4, 12.2)	64.4	4.66, dd (3.0, 12.3)	63.7	4.71, dd (2.6, 11.8)	63.0	4.61, dd (2.6, 11.6)	63.9	5.04, dd (5.7, 12.3)	70.0
14α	1.09, ddd (12.1, 12.2, 12.8)	30.3	1.03, ddd (12.3,12.6,13.2)	31.6	1.27, ddd (11.7, 11.8, 13.2)	29.5	1.60, ddd (11.6,11.9,12.6)	34.6	2.25, ddd (4.2, 5.7, 12.3)	33.8
14β	1.57, ddd (2.4, 3.2, 12.8)		1.42, ddd (3.0, 3.3, 13.2)		1.59, ddd (2.6, 3.2, 13.2)		1.80, ddd (2.6, 3.0,12.6)		2.54, ddd (12.1, 12.2, 12.3)	
15		144.1		147.0		143.6		143.6		139.6
16α	4.96, br s	115.1	4.79, br s	112.5	4.98, br s	115.2	4.98, br s	115.0	5.04, br s	112.3
16β	4.91, br s		4.83, br s		4.92, br s		4.93, br s		5.01, br s	
17	1.70, br s	19.3	1.73, br s	20.1	1.70, br s	18.5	1.72, br s	18.7	1.68, br s	22.6
18α	1.98, br s	20.8	5.55, br s	118.9	1.11, d (7.1)	16.1	1.10, d (6.7)	15.9	1.08, d (6.6)	17.5
18β			5.27, br s							
19	1.95, br s	30.0	1.81, d (1.2)	30.1	1.95, d (1.3)	29.4	1.90, d (1.3)	29.7	1.94, d (1.2)	29.4
20		171.0		171.3		170.8		172.4		171.7
2-OH	1.96, d (6.0)		2.06, d (0.6)		2.10, d (2.6)		2.02, d (1.3)		4.79, br s	
3-OH	2.95, s		2.84, br s		3.00, br s		2.94, br s			

[a] Assignments were aided by $^{1}H-^{1}H$ COSY, spin splitting patterns, analysis of J values, HMBC and HMQC experiments, numbers of attached protons as measured from DEPT spectra, and chemical shift values. [b] Recorded in $CDCl_3$ solution at 500 MHz. The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. [c] Recorded in acetone-d_6 at 300 MHz with δ values referenced to either the residual $(CH_3)_2CO$ (2.04 ppm) or $(CD_3)_2CO$ (29.8 ppm) signals. [d] Recorded in $CDCl_3$ solution at 300 MHz.

clearly correlated with a Dreiding model representing the relative stereochemistry shown in structure **13**.[14]

Undoubtedly, the most striking characteristics of *P. bipinnata* cembranes are their high levels of oxygenation and their diverse pharmacological properties. Many furanocembranolides isolated from this gorgonian (i.e., the bipinnatins) are highly cytotoxic[6] and display inhibitory activity against the nicotinic receptor.[8] Cembrane derivatives closely related to the present bipinnatolides have also been described as potent antiinflammatory agents.[1] Notwithstanding, follow-up biological screening of kallolide A (**1**), bipinnatin J (**4**), bipinnatolide F (**9**), and bipinnatolide G (**10**) in the National Cancer Institute's (NCI) 60-cell-line tumor panel indicated no significant in vitro cancer cell cytotoxicity. On the other hand, bipinnatin H (**7**) and bipinnatin I (**8**) displayed strong cytotoxic action. Of the two compounds, **8** was the more potent, with concentrations of 10^{-6} M eliciting significant differential responses at the GI_{50} level for all the colon and melanoma cancer cell lines. One leukemia cell line [i.e., CCRF-CEM] was substantially more sensitive to compound **8** at a significantly lower concentration ($\log_{10}$ GI_{50} = −7.37). Compound **7** gave a similar pattern in the colon and melanoma tumor subpanels, but required higher concentrations (10^{-4}−10^{-5} M). In an in vitro antituberculosis screen against *Mycobacterium tuberculosis* H37Rv at 12.5 μg/mL kallolide A (**1**), bipinnatin J (**4**), bipinnapterolide A (**5**), and bipinnatolide F (**9**) caused no inhibition. Additional studies to assess the biological properties of these compounds are currently underway.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR were measured at 300 and 75 MHz, respectively, with a General Electric QE-300 spectrometer; and at 500 and 125 MHz, respectively, with a Bruker Avance DRX-500 spectrometer. IR spectra were determined with a Nicolet Magna FT-IR 750 spectrophotometer, and UV spectra were recorded with a Hewlett-Packard Chem Station 8452A spectrometer. The X-ray structures, which were solved by direct methods (SIR92) and completed by successive Fourier calculations, were refined by full-matrix least-squares methods, with anisotropic thermal parameters for all non-H atoms. Following initial refinement, H atoms were located from a difference Fourier map. HO8 was refined with a fixed isotropic thermal parameter and all remaining H atoms were included in the final model at calculated positions, riding on the connected atoms. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corporation.[15] Neutral atom scattering factors were taken from the International Tables for X-ray Crystallography.[16,17] Optical rotations were recorded with a Perkin-Elmer Polarimeter (model 243B) and melting points were determined with a Büchi 535 capillary apparatus and are uncorrected. Column chromatography was performed in Si gel (35−75 mesh) and TLC analyses were carried out using glass precoated Si gel plates. HPLC was performed using a 10 mm Si gel Partisil 10 semipreparative column (9.4 mm × 50 cm). All solvents used were either spectral grade or were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the crude gorgonian extract.

Animal Material. The gorgonian octocoral *P. bipinnata* (Verrill) was collected in May, 1996 near San Andrés Island, Colombia. A voucher specimen (No. PBSAI-01) is stored at the Chemistry Department of the University of Puerto Rico.

Extraction and Isolation. The dry animal (2.1 kg) was blended with MeOH−$CHCl_3$ (1:1) (5 × 1 L), and after filtration, the crude extract was evaporated under vacuum to yield a green residue (167.5 g). After the crude extract was partitioned between hexane and H_2O, the aqueous suspension was extracted with $CHCl_3$ (4 × 1 L). The resulting extract was concentrated in vacuo to yield 43.3 g of an oil which was chromatographed over Si gel (400 g), and separated into 30 fractions (I−XXX) on the basis of TLC analyses. Purification of fraction VIII (8.2 g) by column chromatography over Si gel (400 g) using a step gradient of EtOAc−hexane as eluent afforded 3.76 g (yield = 2.24%) of kallolide A (**1**)[7] and 67.0 mg (yield = 0.04%) of bipinnatin J (**4**).[3j] Purification of fraction X (1.09 g) by size exclusion chromatography on a Bio-Beads SX-3 column using toluene as eluent led to four subfractions. The last subfraction (0.45 g) was purified by normal-phase HPLC [Partisil 10 M9/50 Si gel with 15% 2-propanol in hexane] to afford bipinnatolide F (**9**) (94.0 mg, yield = 0.056%) and bipinnatolide J (**13**) (3.1 mg, yield = 1.85 × 10^{-3}%). Fraction XI (0.98 g) was separated into five subfractions by size exclusion chromatography on a Bio-Beads SX-3 column with toluene as eluent. The last subfraction (0.24 g) was purified by column chromatography over Si gel with 3% acetone in $CHCl_3$ to afford bipinnapterolide A (**5**) (11.1 mg, yield = 6.63 × 10^{-3}%), bipinnatin A (**2**) (4.1 mg, yield = 2.45 × 10^{-3}%),[6] bipinnatin C (**3**) (2.0 mg, yield = 1.19 × 10^{-3}%),[6] bipinnatin G (**6**) (4.2 mg, yield = 2.51 × 10^{-3}%), bipinnatolide H (**11**) (5.6 mg, yield = 3.34 × 10^{-3}%), and a mixture containing compound **12**. The latter was further purified by normal-phase HPLC [Partisil 10 M9/50 Si gel with 15% 2-propanol in hexane] to yield pure bipinnatolide I (**12**) (9.6 mg, yield = 5.73 × 10^{-3}%). Fraction XII (0.57 g) was separated into five subfractions by size exclusion chromatography on a Bio-Beads SX-3 column with toluene as eluent. The last subfraction (0.24 g) was purified by column chromatography over Si gel with 3% acetone in $CHCl_3$ to yield bipinnatolide G (**10**) (14.3 mg, yield = 8.54 × 10^{-3}%). Fraction XIV (1.51 g) was separated into four subfractions by size exclusion chromatography on a Bio-Beads SX-3 column using toluene as eluent. The fourth subfraction was purified by column chromatography over Si gel with 4% acetone in $CHCl_3$ to yield bipinnatin H (**7**) (41.2 mg, yield = 2.46 × 10^{-2}%) and bipinnatin I (**8**) (58.9 mg, yield = 3.52 × 10^{-2}%).

Bipinnapterolide A (5): colorless crystals, mp 153−154 °C; $[\alpha]^{24}_D$ −7.8° (*c* 2.1, $CHCl_3$); IR (film) 3551, 3074, 1756, 1718, 1706, 1646, 1264, 1121, 1067, 908 cm^{-1}; UV λ_{max} (MeOH) 216 nm (ϵ 9800); ^{1}H NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* $[M - C_2HO_2]^+$ 303 (3), 289 (5), 218 (10), 201 (10), 173 (15), 167 (15), 164 (45), 151 (32), 150 (100), 139 (31), 135 (22), 105 (30), 97 (33), 96 (44), 85 (44), 81 (39), 69 (30); HRFABMS *m*/*z* $[M + Na]^+$ 383.1468 (calcd for $C_{20}H_{24}O_6Na$, 383.1471).

Single-Crystal X-ray Diffraction Analysis of Bipinnapterolide A·H_2O. Crystallization of bipinnapterolide A (**5**) by slow evaporation from EtOAc yielded colorless needle crystals of excellent quality. A specimen of 0.24 × 0.16 × 0.08 mm was selected for the analysis and mounted on a glass fiber. X-ray diffraction data were collected on a Siemens SMART CCD system at 23 ± 1 °C to a maximum 2θ of 53.9°, using Mo Kα radiation (λ = 0.710 69 Å). Preliminary X-ray photographs showed monoclinic symmetry, and accurate lattice constants of *a* = 10.0500(9) Å, *b* = 10.1699(9) Å, and *c* = 10.1612(9) Å. The systematic extinctions, crystal density (d_{calc} = 1.302 g/cm^3), and optical activity indicated space group $P2_1$ in the asymmetric unit (*Z* = 2) of composition $C_{20}H_{26}O_7$ with formula weight of 378.42. Of the 5149 reflections measured, 2039 were unique (R_{int} = 0.020); equivalent reflections were merged. The crystallographic residual was *R* = 4.3% (R_w = 5.5%) for the observed reflections.

Bipinnatin A (2) and bipinnatin C (3): physical and spectroscopic properties of metabolites **2** and **3** have been previously described in the literature.[6]

Bipinnatin G (6): white solid; $[\alpha]^{24}_D$ −12.9° (*c* 2.1, $CHCl_3$); IR (film) 3077, 2929, 1753, 1734, 1652, 1375, 1249, 1077, 1020, 955 cm^{-1}; UV λ_{max} (MeOH) 212 nm (ϵ 18 000); ^{1}H NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* $[M]^+$ 444 (23), 385 (13), 384 (21), 342 (31), 324 (25), 284 (9), 230 (26), 180 (84), 177 (22), 166 (24), 163

(26), 140 (27), 139 (100), 138 (39), 137 (70), 124 (27), 91 (38), 65 (37); HREIMS *m/z* $[M]^+$ 444.1785 (calcd for $C_{24}H_{28}O_8$, 444.1784).

Bipinnatin H (7): colorless crystals, mp 221−222 °C; $[\alpha]^{24}_D$ −8.8° (*c* 2.1, $CHCl_3$); IR (film) 3083, 2935, 1757, 1736, 1376, 1236, 1095, 1028, 955, 753 cm^{-1}; UV λ_{max} (MeOH) 214 nm (ϵ 19 000); ^1H NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; EIMS *m/z* $[M]^+$ 460 (34), 418 (13), 398 (11), 358 (35), 357 (18), 341 (38), 340 (67), 332 (14), 327 (70), 297 (43), 248 (26), 245 (24), 241 (25), 219 (36), 201 (28), 180 (56), 179 (43), 178 (38), 177 (31), 167 (46), 163 (48), 140 (40), 139 (100), 138 (30), 137 (84), 124 (44), 123 (65), 121 (44), 93 (60), 91 (80), 70 (99), 65 (66); HREIMS *m/z* $[M]^+$ 460.1717 (calcd for $C_{24}H_{28}O_9$, 460.1733).

Bipinnatin I (8): colorless gum; $[\alpha]^{24}_D$ −8.5° (*c* 1.2, $CHCl_3$); IR (film) 3084, 2933, 1756, 1740, 1625, 1238, 1154, 1093, 1029, 958 cm^{-1}; UV λ_{max} (MeOH) 212 nm (ϵ 14 600); ^1H NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; EIMS *m/z* $[M]^+$ 488 (6), 446 (2), 428 (3), 386 (18), 369 (16), 368 (25), 355 (11), 274 (15), 222 (17), 181 (12), 180 (100), 179 (17), 173 (12), 140 (20), 139 (48), 138 (18), 137 (43), 124 (11), 93 (19), 91 (19), 77 (14), 65 (22); HREIMS *m/z* $[M]^+$ 488.1687 (calcd for $C_{25}H_{28}O_{10}$, 488.1682).

Bipinnatolide F (9): colorless crystals, mp 204−208 °C dec; $[\alpha]^{24}_D$ +25.3° (*c* 0.95, $CHCl_3$); IR (film) 3575, 3564, 3364, 3087, 2966, 2929, 1782, 1670, 1608, 1441, 1287, 1100, 1044 cm^{-1}; UV λ_{max} (MeOH) 204 nm (ϵ 7700), 244 nm (ϵ 11 800); ^1H NMR (500 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (125 MHz, $CDCl_3$), see Table 2; EIMS *m/z* $[M]^+$ 376 (1), 358 (53), 275 (6), 192 (9), 190 (10), 180 (59), 179 (95), 175 (20), 152 (16), 151 (100), 150 (56), 149 (16), 147 (21), 135 (29), 123 (31), 122 (52), 121 (24), 113 (52), 109 (48), 97 (20), 84 (65), 83 (51), 81 (47), 79 (41), 69 (43); HREIMS *m/z* $[M]^+$ 376.1517 (calcd for $C_{20}H_{24}O_7$, 376.1522).

Single-Crystal X-ray Diffraction Analysis of Bipinnatolide F. Crystallization of bipinnatolide F (**9**) by slow evaporation from EtOAc yielded colorless needle crystals of excellent quality. A specimen of 0.31 × 0.11 × 0.06 mm was selected for the analysis and mounted on a glass fiber. X-ray diffraction data were collected on a Siemens SMART CCD system at 23 ± 1 °C to a maximum 2θ of 54.0°, using Mo Kα radiation (λ = 0.710 69 Å). Preliminary X-ray photographs showed monoclinic symmetry, and accurate lattice constants of a = 11.658(1) Å, b = 6.1675(5) Å, and c = 14.256(1) Å. The systematic extinctions, crystal density (d_{calc} = 1.330 g/cm^3), and optical activity indicated space group $P2_1$ in the asymmetric unit (Z = 2) of composition $C_{20}H_{24}O_7$ with formula weight of 376.41. Of the 4935 reflections measured, 2058 were unique (R_{int} = 0.019); equivalent reflections were merged. The crystallographic residual was R = 4.7% (R_w = 7.7%) for the observed reflections.

Bipinnatolide G (10): colorless crystals; mp 234−235 °C; $[\alpha]^{24}_D$ +3.9° (*c* 0.51, $CHCl_3$); IR (film) 3508, 3289, 3103, 3082, 2976, 2930, 1783, 1674, 1617, 1442, 1256, 1139, 1109, 1043 cm^{-1}; UV λ_{max} (MeOH) 206 nm (ϵ 6200), 242 nm (ϵ 10 900); ^1H NMR (300 MHz, acetone-d_6), see Table 2; ^{13}C NMR (75 MHz, acetone-d_6), see Table 2; HRFABMS *m/z* $[M + Li]^+$ 383.1668 (calcd for $C_{20}H_{24}O_7Li$, 383.1682).

Bipinnatolide H (11): colorless crystals, mp 205−206 °C; $[\alpha]^{24}_D$ +10.0° (*c* 1.40, $CHCl_3$); IR (film) 3485, 3473, 3328, 3070, 2975, 2937, 1783, 1670, 1618, 1434, 1291, 1154, 1088, 1059 cm^{-1}; UV λ_{max} (MeOH) 206 nm (ϵ 4700), 240 nm (ϵ 10 400); ^1H NMR (300 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 2; HRFABMS *m/z* $[M + Na]^+$ 401.1574 (calcd for $C_{20}H_{26}O_7Na$, 401.1576).

Bipinnatolide I (12): colorless crystals, mp 162−163 °C dec; $[\alpha]^{24}_D$ +22.9° (*c* 2.4, $CHCl_3$); IR (film) 3537, 3464, 3108, 3083, 2924, 1744, 1670, 1615, 1451, 1083, 1054, 900 cm^{-1}; UV λ_{max} (MeOH) 228 nm (ϵ 12 500); ^1H NMR (300 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 2; EIMS *m/z* $[M - H_2O]^+$ 344 (20), 316 (26), 265 (24), 233 (28), 192 (18), 154 (49), 152 (100), 135 (35), 109 (57), 91 (41), 83 (86), 82 (85), 69 (44), 55 (38); HRFABMS *m/z* $[M + Na]^+$ 385.1626 (calcd for $C_{20}H_{26}O_6Na$, 385.1627).

Bipinnatolide J (13): colorless crystals, mp 180−183 °C dec; $[\alpha]^{24}_D$ +3.9° (*c* 1.55, $CHCl_3$); IR (film) 3403, 3091, 2976, 2944, 1746, 1730 (sh), 1677, 1654, 1625, 1350, 1124, 1022, 920 cm^{-1}; UV λ_{max} (MeOH) 212 nm (ϵ 8000); ^1H NMR (300 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (75 MHz, $CDCl_3$), see Table 2; HRFABMS *m/z* $[M + Na]^+$ 383.1470 (calcd for $C_{20}H_{24}O_6Na$, 383.1471).

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(10) Selected correlations observed in the 2D NOE spectrum of bipinnatin I (**8**) were H-1 [H-14′]; H-2 [H-10, Me-18]; H-5 [Me-18, Me-19]; H-7 [H-9]; H-9′ [H-10, Me-19]; H-11 [H-10, H- 13, Me-19].
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(13) Another conceivable structure for bipinnatolide I (**12**) was carefully considered in which the hemiacetal functionality was placed at C-2 and the secondary alcohol at C-3. However, such an alternative structure was dismissed readily from the $^1H-^1H$ COSY and HMBC NMR data alone.
(14) Selected correlations observed in the 2D NOE spectrum of bipinnatolide J (**13**) were H-1 [H- 13, H-14β]; H-4 [H-11, Me-18]; H-5α [H-5β, H-7, Me-18]; H-7 [Me-19]; H-9α [H-9β, H- 10, Me-19]; H-9β [H-11]; H-11 [H-14β]; H-13 [H-14β]; H-14α [Me-17, Me-19]; and Me-18 [H-16β].
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