Bipinnatins K–Q, Minor Cembrane-Type Diterpenes from the West Indian Gorgonian *Pseudopterogorgia kallos*: Isolation, Structure Assignment, and Evaluation of Biological Activities[⊥]

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An extensive chemical study of the secondary metabolites found in the crude organic extract of the gorgonian octocoral *Pseudopterogorgia kallos* has led to the isolation of seven new cembranolides, bipinnatins K–Q (**2**–**8**), and one known compound, bipinnatin E (**9**). The molecular structures of compounds **2**–**8**, many of which contain unusual structural features, were assigned mainly by 2D NMR spectroscopic methods and X-ray crystallographic analysis. The discovery of compounds **2**–**8** may lend support to previously proposed mechanisms for the biosynthesis of recently isolated bioactive natural products from the same gorgonian specimen. The in vitro cytotoxicity of bipinnatins **4**, **6**, and **7** against the NCI tumor cells MCF breast cancer, NCI-H460 non-small cell lung cancer, and SF-268 CNS cancer was evaluated. However, only bipinnatin Q (**6**) displayed significant cytotoxic activity. Some of the compounds isolated proved to be inhibitors of acetylcholine receptors.

The gorgonian coral genus *Pseudopterogorgia* is comprised of over 20 species, most of which can be found in the West Indies region from Bermuda to the Bahamas, the Florida Keys, the Greater and Lesser Antilles, and the northern coast of South America to Brazil.[1] *Pseudopterogorgia* species, which are best characterized as "sea plumes" based upon their large, highly plumose and physically soft forms, are well-known for the production of diterpenoids of fascinating molecular architecture that exhibit a wide spectrum of biological activities including antibacterial, antiinflammatory, antimalarial, and cytotoxic properties.[2] An early investigation on the chemical composition of *Pseudopterogorgia kallos* (Bielschowsky, 1918) showed that it is a rich source of pseudopterane diterpenoids.[3] However, during the last five years (2003–2008) subsequent chemical scrutiny has demonstrated that this gorgonian species also contains a number of minor bioactive diterpenes that are based on distinctively novel carbon frameworks (i.e., bielschowskysin, ciereszkolide, intricarene, kallosin A, and providencin).[4] In the present study, we report the isolation, structure assignment, and biological evaluation of seven new minor cembranolides, named bipinnatins K–Q (**2**–**8**),[5] most of which appear to originate biosynthetically from the known furanocembranolide bipinnatin J (**1**)[6] or its undiscovered $\Delta^{7(8)}$ *E*- geometric isomer. The structures were determined mainly through the use of 1D and 2D NMR techniques. For compounds **3** and **5** the deductions were confirmed by X-ray crystallographic analysis. In the course of this investigation we also isolated the known compound bipinnatin E (**9**). Herein we report for the first time the complete physical and chemical data for this substance. Interestingly, this is the only report thus far that describes the isolation of cembranoid diterpenes from this gorgonian species.

Results and Discussion

Bipinnatin K (**2**) displayed a molecular ion peak at *m/z* 446 in the EIMS. This information, together with data from a HRFABMS analysis and the ^{13}C NMR DEPT spectrum, suggested the molecular formula to be $C_{23}H_{26}O_9$. The IR spectrum showed absorption bands at 3510 (hydroxyl group), 1761 (α,β-unsaturated-γ-lactone), 1736 (acetate), and 962 cm^{-1} (exomethylene with electron-withdrawing group attached), and the UV spectrum revealed the characteristic UV absorption of a conjugated γ-butyrolactone.[7] The ^{1}H NMR spectrum of **2** indicated the presence of four methyl groups with three-proton singlets at δ 3.35, 2.22, 2.04, and 1.32 ppm. It also exhibited two doublets with fine splitting at δ 6.37 (1H, J = 3.5 Hz) and 5.71 (1H, J = 3.1 Hz) ascribed to the exomethylene group of a α-methylene-γ-lactone ring. A set of additional olefinic proton signals at δ 6.32 (s) and 5.91 (brd, J = 0.9 Hz), each integrating for one proton, pointed to the presence of an α,α′,β-trisubstituted furan and an α,γ-disubstituted-γ-butyrolactone ring moiety, respectively, as found in bipinnatin J (**1**). Appropriate ^{13}C NMR signals were observed at δ 9.7 (C-18), 78.4 (C-10), 111.6 (C-5), 124.3 (C-4), 128.3 (C-12), 143.8 (C-3), 152.3 (C-6), 156.0 (C-11), and 169.6 (C-20). In the ^{1}H NMR spectrum of **2**, the doublet of doublets at δ 5.27 (J = 12.4, 4.7 Hz), the doublet at δ 5.15 (J = 8.9 Hz), the doublet of a doublet of doublets at δ 4.96 (J = 11.8, 5.8, 0.9 Hz), and the singlet at δ 3.96 were attributable to the oxymethines H-13, H-2, H-10, and H-7, respectively. The HSQC spectrum confirmed that these protons were bonded to oxygen-bearing carbons at δ_C 65.7, 74.0, 78.4, and 85.5, respectively.

Correlations deduced from extensive analyses of the 2D NMR data of **2** (^{1}H–^{1}H COSY, TOCSY, HSQC, and HMBC NMR experiments) in $CDCl_3$ enabled initially the establishment of four partial structures (**a**–**d**) that were subsequently interconnected to yield the final structure of bipinnatin K (see Figure 1). The ^{1}H and ^{13}C NMR data are presented in Tables 1 and 2.

The relative configuration of bipinnatin K (**2**) was established from the splitting patterns and coupling constants of H-2 (d, J = 8.9 Hz), H-13 (dd, J = 12.4 and 4.7 Hz), and H-10 (ddd, J = 11.8, 5.8, 0.9 Hz) and the lack of significant coupling between H-10 and H-11.[7] In addition, NOESY correlations between H-7 and H-9β, H-2 and H_3-18, H-2 and H-14α, H-11 and H-13, H-11 and H_3-19, H-9α and H-10, H-10 and H_3-19, H-5 and H_3-18, H_3-19 and H_3-21, and H-1 and H-14β supported the proposed relative stereochemistry of **2** (see computer-generated 3D drawing in Figure 2). It is conceivable that bipinnatin K might be an isolation artifact, and its presence in the crude gorgonian extract implies that the true natural product present in this animal possesses a highly reactive furanyl epoxide array (as in **9**) that is prone to S_N2-type methanolysis at C-7. Such a transient epoxide would also be the most plausible biogenetic precursor to the rearranged furanocembrane bielschowskysin[4c] (see Supporting Information).

[⊥] Dedicated to Dr. G. Robert Pettit of Arizona State University for his pioneering work on bioactive natural products.

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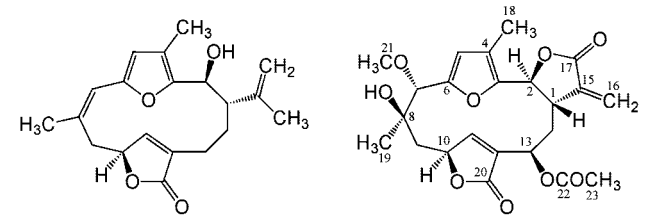
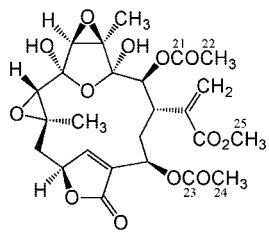
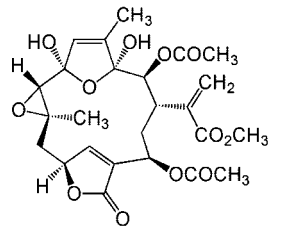


bipinnatin J (**1**)

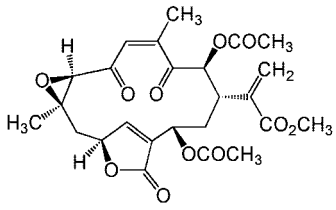
bipinnatin K (**2**)



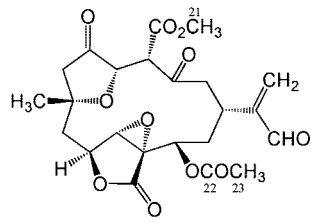
bipinnatin L (**3**)



bipinnatin M (**4**)



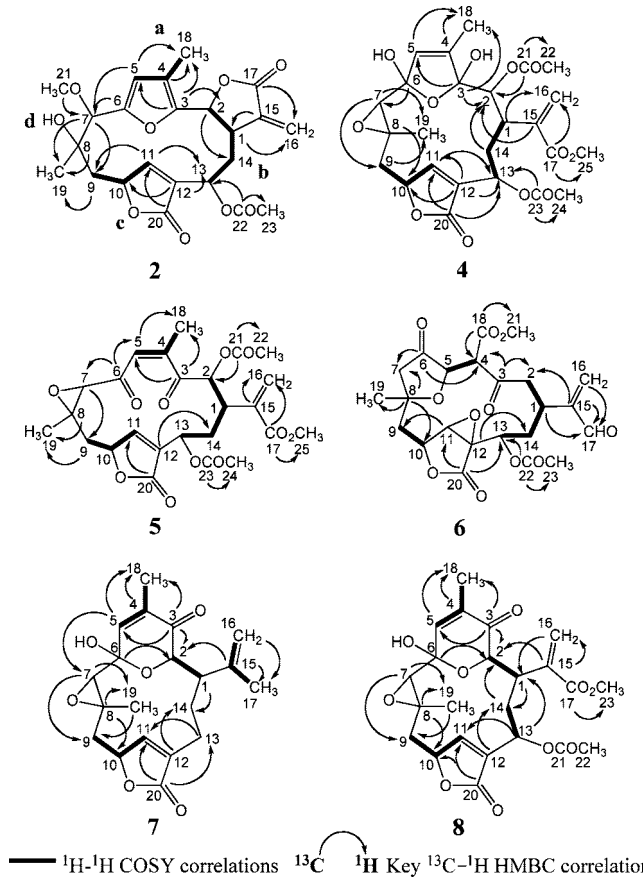
bipinnatin P (**5**)



bipinnatin Q (**6**)

Bipinnatin L (**3**) was isolated as a UV-active, yellowish oil. The HRESIMS showed a sodiated molecular ion at *m/z* 561.1561 [M + Na]$^+$, corresponding to the molecular formula $C_{25}H_{30}O_{13}Na$. The IR spectrum showed the presence of hydroxyl (3435 cm^{-1}) and carbonyl (1750 and 1717 cm^{-1}) groups. The ^{1}H NMR spectrum of **3** displayed distinctive signals at δ 3.37 (s, 1H), 3.47 (s, 1H), 1.50 (s, 3H), and 1.56 (s, 3H), which together accounted for two methyl-bearing trisubstituted epoxide moieties. Other ^{1}H NMR signals suggested that two secondary acetates and an α,γ-disubstituted-α,β-unsaturated-γ-butyrolactone (as was the case for compound **2**) were present in **3**. The ^{13}C NMR spectrum showed signals that correlated easily with those of the proposed α,α'-dihydroxy-β,β'-epoxy-β-methyl furan moiety of compound **3**.[8] Except for the additional acetate and carbomethoxyl groups, the number and types of the ^{1}H and ^{13}C NMR signals (Tables 1 and 2) associated with the cembranoid macrocycle were essentially the same as those of compound **2**, with only a few major differences in their chemical shifts. The complete analysis of the correlations observed in the ^{1}H–^{1}H COSY, TOCSY, and HMBC spectra permitted us to assign all the spectroscopic signals and to propose the planar structure for compound **3**. Finally, the detailed structure and relative stereochemistry of **3** were established unambiguously from single-crystal X-ray analysis (ORTEP drawing in Figure 3). Observation of an NOE between H-1/H-2, H-1/H-7, H-2/H-13, H-2/H-14α, H-7/H-9β, H-9α/H-10, H-9α/H$_3$-19, H-10/H-11, H-11/H-13, and H-11/H$_3$-19 indicated 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-11 or between protons H-1 and H$_3$-18 confirmed that the solution conformation is similar to that in the solid state as elucidated by X-ray analysis.

The major compound isolated, bipinnatin M (**4**), showed a sodiated pseudomolecular ion peak at *m/z* 545.1631 [M + Na]$^+$ in the HRESIMS, consistent with a molecular formula of $C_{25}H_{30}O_{12}Na$, one oxygen atom less than in the molecular formula of bipinnatin L (**3**). IR absorptions implied the presence of hydroxyl (3534 and 3388 cm^{-1}) and ester carbonyl (1754, 1742, 1728, and 1719 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR (Tables 1 and 2) spectra of **4** were generally analogous to those of **3** except for the following



2 **4** **5** **6** **7** **8**

^{1}H-^{1}H COSY correlations ^{13}C ^{1}H Key ^{13}C–^{1}H HMBC correlation

Figure 1. ^{1}H−^{1}H COSY and HMBC correlations for compounds **2** and **4**−**8**.

observation: two sp^2 carbons [δ_C 131.0 (CH) and 140.3 (C)] were observed in place of the two sp^3 carbons [δ_C 62.2 (CH) and 64.8 (C)] bearing an oxygen atom of **3**. The positions of these olefinic carbons were deduced to be C-4 and C-5 by HMBC correlations for H-2 and H$_3$-18 to C-4, and H-7 and H$_3$-18 to C-5. Further analysis of the correlations observed in the ^{1}H–^{1}H COSY, TOCSY, and HMBC spectra allowed us to confidently assign all of the signals and to propose the planar structure for **4** (Figure 1). The relative stereochemistry of **4** was deduced from NOESY correlations, the splitting patterns, and coupling constants of H-9α (dd, J = 13.0, 8.2 Hz), H-9β (dd, J = 13.0, 9.0 Hz), H-10 (brt, J = 8.4 Hz), and H-13 (brd, J = 8.2 Hz), and most importantly, the unexpected lack of coupling between H-1 and H-2. In the NOESY spectrum of **4**, NOEs were observed between the H$_3$-19 methyl at δ 1.71 and the protons at H-9α (δ 2.96) and H-11 (δ 7.89). Key NOEs were also observed between H-1 at δ 3.37 (signal overlapped with H-7) and the protons at H-2 (δ 5.45) and H-11. The latter proton, in turn, showed a strong NOE with H-10, and H-7 showed a strong NOE cross-peak with H-9β. The combination of these NMR results with those of molecular modeling calculations (all 16 stereoisomeric structures involving the C-1,2,3,6 chiral array were drawn) led to the conclusion that the only compatible 3D structure was that displaying the C-3,6 hydroxyls along with the alkenyl and methyl side groups at C-1 and C-8, respectively, in an all-*cis* arrangement (with α-orientation, see Figure 2).[9] The other conceivable stereoisomers were compatible with neither the NOEs nor with most of the proton–proton coupling constants experimentally observed. Chemical shifts found for compound **4** are in agreement with those reported for similar structural arrangements present in bipinnatins.[10,11] Several attempts were made to correlate chemically the structures of compounds **3** and **4** upon oxidation of bipinnatin M (**4**) using both *m*-CPBA and MMPP under a variety of reaction conditions. However, in each instance, unreacted

Table 1. ^{1}HNMR (500 MHz) Spectral Data for Bipinnatins K−M (**2**−**4**) and Bipinnatins P−Q (**5**, **6**)[a]

	δ_H, mult, intrgt (*J* in Hz)				
atom	bipinnatin K (**2**)	bipinnatin L (**3**)	bipinnatin M (**4**)	bipinnatin P (**5**)	bipinnatin Q (**6**)
1	3.15, 1H, m	3.70, 1H, m	3.37, 1H, brs	2.96, 1H, dt (12.1, 3.4)	3.54, 1H, m
2	5.15, 1H, d (8.9)	5.16, 1H, d (4.7)	5.45, 1H, s	6.21, 1H, d (3.4)	2.57, 2H, brm
3					
4					3.97, 1H, d (2.9)
5	6.32, 1H, s	3.47, 1H, s	5.68, 1H, brd (1.1)	6.47, 1H, q (1.7)	4.84, 1H, d (2.9)
6					
7α	3.96, 1H, s	3.37, 1H, s	3.37, 1H, brs	3.40, 1H, s	2.65, 1H, d (18.3)
7*β*					2.54, 1H, d (18.3)
8					
9α	2.65, 1H, dd (14.8, 5.8)	2.58, 1H, dd (14.7, 5.4)	2.96, 1H, dd (13.0, 8.2)	3.01, 1H, dd (13.3, 4.6)	2.31, 1H, dd (15.4, 2.1)
9*β*	1.76, 1H, dd (14.8, 11.8)	1.93, 1H, m	1.27, 1H, dd (13.0, 9.0)	1.38, 1H, t (12.7)	2.55, 1H, m
10	4.96, 1H, ddd (11.8, 5.8, 0.9)	5.15, 1H, brt (4.5)	5.19, 1H, brt (8.4)	5.13, 1H, ddd (12.0, 4.5, 1.4)	4.75, 1H, dd (5.6, 2.4)
11	5.91, 1H, brd (0.9)	7.47, 1H, brd (0.9)	7.89, 1H, s	7.51, 1H, d (1.3)	4.33, 1H, s
12					
13	5.27, 1H, dd (12.4, 4.7)	5.67, 1H, dd (7.3, 2.6)	5.73, 1H, brd (8.2)	5.53, 1H, dd (11.8, 5.0)	5.41, 1H, dd (9.9, 4.5)
14α	3.05, 1H, dt (12.7, 3.7)	1.84, 1H, ddd (14.7, 4.9, 2.9)	2.31, 1H, brm	1.88, 1H, ddd (13.1, 12.4, 5.1)	2.11, 1H, ddd (14.3, 10.0, 4.5)
14*β*	1.94, 1H, dt (12.7, 4.7)	2.84, 1H, brs	2.10, 1H, brm	2.42, 1H, ddd (13.2, 12.0, 3.2)	2.43, 1H, ddd (14.3, 9.8, 4.1)
15					
16α	6.37, 1H, d (3.5)	6.34, 1H, d (1.1)	6.39, 1H, brs	6.32, 1H, brs	6.39, 1H, s
16*β*	5.71, 1H, d (3.1)	6.09, 1H, brs	5.80, 1H, brs	6.12, 1H, brs	6.15, 1H, s
17					9.45, 1H, s
18	2.22, 3H, s	1.56, 3H, s	1.71, 3H, s	1.82, 3H, d (1.7)	
19	1.32, 3H, s	1.50, 3H, s	1.71, 3H, s	1.56, 3H, s	1.51, 3H, s
20					
21	3.35, 3H, s				3.73, 3H, s
22	2.04, 3H, s	2.04, 3H, s	2.19, 3H, s	2.02, 3H, s	
23					2.06, 3H, s
24		1.96, 3H, s	1.93, 3H, s	2.34, 3H, s	
25		3.73, 3H, s	3.69, 3H, s	3.75, 3H, s	
−OH	2.90, 1H, brs				

[a] NMR spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are given in ppm and are referenced to the residual $CHCl_3$ signal (δ_H 7.26).

Table 2. ^{13}C NMR (125 MHz) Spectral Data for Bipinnatins K–M (**2**–**4**) and Bipinnatins P–Q (**5**, **6**)[a]

	δ_C (mult)				
atom	bipinnatin K (**2**)	bipinnatin L (**3**)	bipinnatin M (**4**)	bipinnatin P (**5**)	bipinnatin Q (**6**)
1	39.2 (CH)	36.5 (CH)[b]	32.3 (CH)[c]	36.5 (CH)	29.3 (CH)
2	74.0 (CH)	76.2 (CH)	77.6 (CH)	74.4 (CH)	45.7 (CH_2)
3	143.8 (C)	103.0 (C)	109.3 (C)	201.9 (C)	202.1 (C)
4	124.3 (C)	64.8 (C)	140.3 (C)	155.8 (C)	61.3 (CH)
5	111.6 (CH)	62.2 (CH)	131.0 (CH)	122.2 (CH)	75.8 (CH)
6	152.3 (C)	99.8 (C)	105.6 (C)	195.5 (C)	211.7 (C)
7	85.5 (CH)	58.5 (CH)	63.1 (CH)	64.9 (CH)	50.5 (CH_2)
8	72.7 (C)	58.7 (C)	59.2 (C)	60.6 (C)	80.1 (C)
9	41.8 (CH_2)	42.0 (CH_2)	44.3 (CH_2)	39.6 (CH_2)	41.9 (CH_2)
10	78.4 (CH)	77.2 (CH)	77.5 (CH)	77.8 (CH)	77.9 (CH)
11	156.0 (CH)	151.0 (CH)	152.7 (CH)	153.8 (CH)	66.6 (CH)
12	128.3 (C)	133.2 (C)	130.7 (C)	130.2 (C)	59.9 (C)
13	65.7 (CH)	67.7 (CH)	66.5 (CH)	66.5 (CH)	67.3 (CH)
14	32.9 (CH_2)	37.0 (CH_2)	37.7 (CH_2)[b]	31.6 (CH_2)	34.1 (CH_2)
15	137.5 (C)	140.0 (C)[c]	139.1 (C)	139.4 (C)	152.0 (C)
16	122.4 (CH_2)	129.0 (CH_2)[c]	129.3 (CH_2)	128.8 (CH_2)	135.6 (CH_2)
17	168.5 (C)	167.5 (C)	167.2 (C)	166.1 (C)	193.6 (CH)
18	9.7 (CH_3)	13.1 (CH_3)	12.9 (CH_3)	23.0 (CH_3)	167.7 (C)
19	19.0 (CH_3)	19.8 (CH_3)[b]	17.2 (CH_3)	23.1 (CH_3)	26.3 (CH_3)
20	169.6 (C)	169.5 (C)	169.9 (C)	168.6 (C)	168.8 (C)
21	58.0 (CH_3)	171.2 (C)	171.5 (C)	170.0 (C)	52.6 (CH_3)
22	170.3 (C)	20.7 (CH_3)	20.9 (CH_3)	20.9 (CH_3)	169.8 (C)
23	21.0 (CH_3)	170.5 (C)	170.1 (C)	169.7 (C)	20.8 (CH_3)
24		20.6 (CH_3)	20.6 (CH_3)	20.6 (CH_3)	
25		51.6 (CH_3)	52.4 (CH_3)	52.6 (CH_3)	

[a] NMR spectra were recorded in $CDCl_3$ at 25 °C. ^{13}C NMR chemical shift values are in ppm and were referenced to the $CDCl_3$ (δ_C 77.0) signal. ^{13}C NMR multiplicities were deduced from DEPT NMR experiments. [b] Low-intensity (broad) carbon resonance. [c] Undetected carbon resonance line. The chemical shift value shown was estimated from HMBC and/or HSQC NMR experiments.

bipinnatin M was always recovered. No further attempts were made to correlate the structures of these natural products, as large amounts of pure compound **4** were required for evaluating its biological properties. Unfortunately, all attempts to recrystallize **4** using a variety of organic solvents led to crystals that did not diffract X-rays.

Compound **5**, named bipinnatin P, was isolated as a UV-active, colorless, crystalline solid. It showed a sodiated pseudomolecular ion at *m*/*z* 527.1521 $[M + Na]^+$ in the HRESIMS, in agreement with a molecular formula of $C_{25}H_{28}O_{11}Na$. The IR spectrum showed carbonyl (1767, 1714, 1678 cm^{-1}), olefin (1625 cm^{-1}), and epoxide (1235 cm^{-1}) bands. The ^{13}C NMR spectrum exhibited six carbonyl signals at δ_C 201.9, 195.5, 170.0, 169.7, 168.6, and 166.1 along with signals associated with a methyl-bearing trisubstituted epoxide [δ_C 64.9 (CH), 60.6 (C), 23.1 (CH_3)]. The ^{1}H NMR spectrum

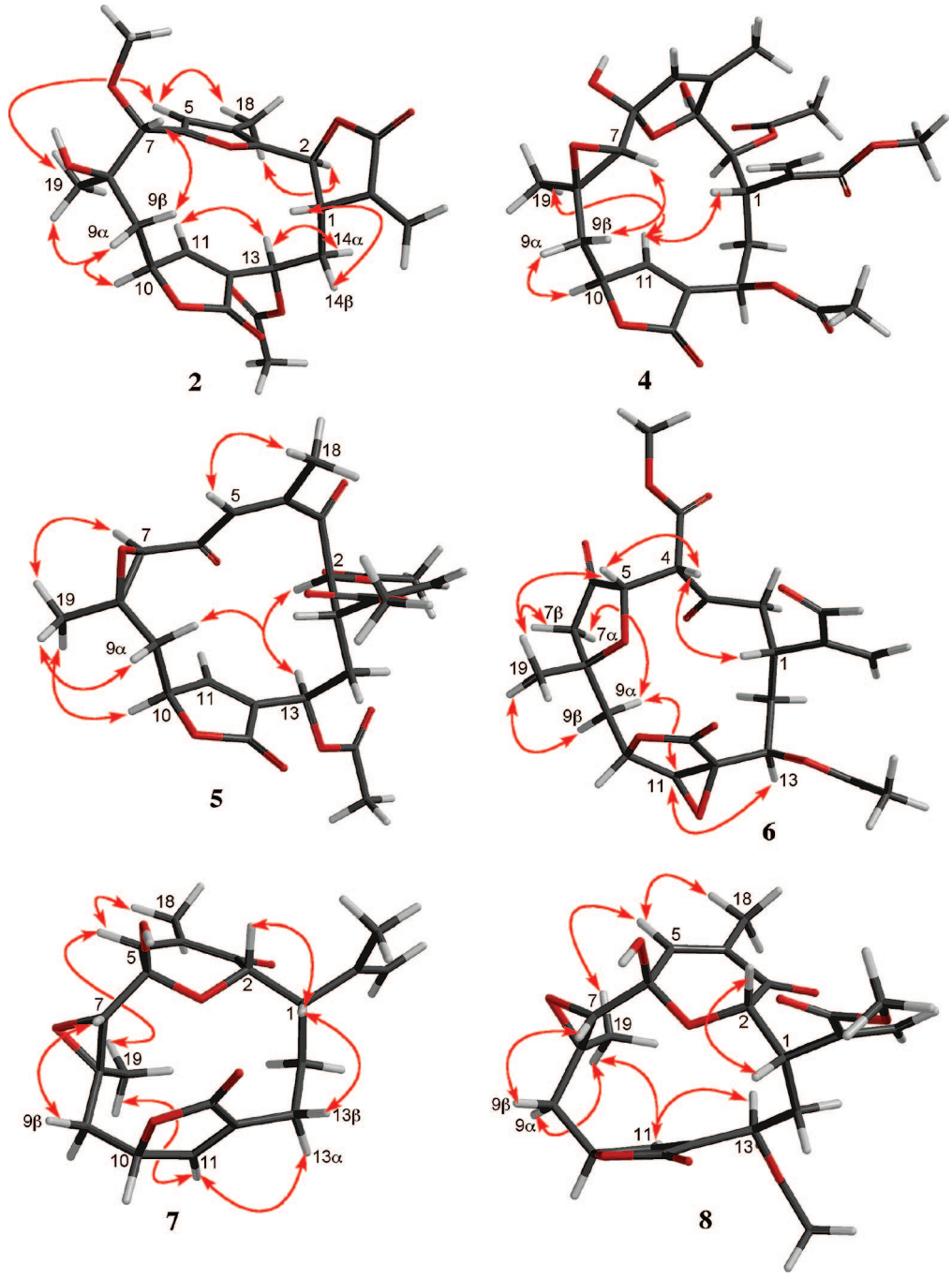


Figure 2. Selected NOESY correlation of compounds **2** and **4**–**8**.

showed four singlets at δ_H 3.75, 2.34, 2.02, and 1.56 and a doublet with fine splitting at δ 1.82 (J = 1.7 Hz), corresponding to five methyl groups; it also showed two broad singlets at δ 6.32 and 6.12, corresponding to a conjugated methylidene. Finally, resonances appeared at δ 6.47 (q, 1H, J = 1.7 Hz) and 7.51 (d, 1H, J = 1.3 Hz) for two conjugated olefinic protons. The number and types of the ^{1}H and ^{13}C NMR signals associated with the bipinnatin macrocyclic system were essentially the same as those of compound **4**, with major differences observed only in the chemical shifts of carbons C-3, C-5, and C-18. The complete analysis of the correlations observed in the ^{1}H–^{1}H COSY and HMBC spectra allowed us to assign all of the signals and to propose the planar structure for enedione **5** (Figure 1).

The relative configuration of bipinnatin P (**5**) was determined by a NOESY experiment. Thus, the *Z*-geometry for the $\Delta^{4(5)}$ trisubstituted olefin and that of the trisubstituted epoxide was revealed from strong correlations from H-7 to H_3-19 and from H-5 to H_3-18. Additional NOEs were observed among H-11 and both H-2 and H-13, and since NOE cross-peaks were also detected between H-2 and H-13, the latter sets of protons were arranged spatially on the bottom face of the molecule. NOESY correlations between H-9α (δ 3.01) with both H_3-19 (δ 1.56) and H-10 (δ 5.13) showed that these protons are all α as drawn, and a NOESY cross-peak between H-9β (δ 1.38) and H-1 (δ 2.96) demonstrated that the alkylidene side chain functionality is α at C-1, as shown in **5** (Figure 2). These results established the acetate groups at C-2 and C-13 as having a β-equatorial orientation. The relative configuration of bipinnatin P (**5**) was further confirmed by X-ray crystallographic analysis (Figure 3). An attempt to dehydrate bipinnatin M (**4**) under acid-catalyzed conditions into its corresponding enedione derivative (like **5** but with *E* configuration about the epoxide) led instead to the formation of an intractable mixture of products.

Bipinnatin Q (**6**) was obtained as a UV-active, yellowish oil, and its molecular formula $C_{23}H_{26}O_{11}$ was established by HRESIMS

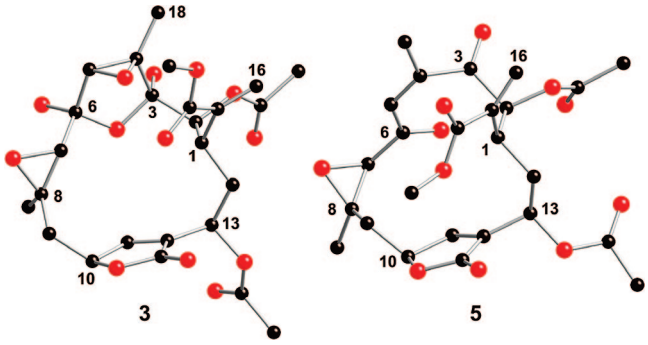
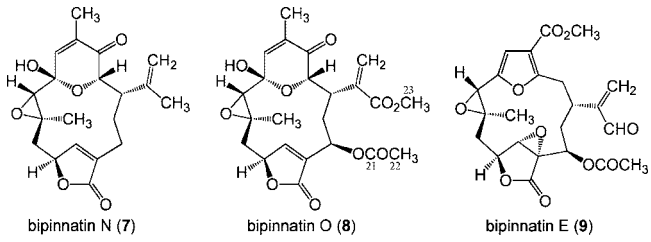


Figure 3. Illustration of the X-ray crystal structures of bipinnatin L (**3**) and bipinnatin P (**5**). The crystallographic models show the atom-numbering scheme and the relative stereochemistry.

([M + Na]$^+$, *m/z* 501.1368, calcd 501.1373). The IR spectrum showed strong absorption peaks at 1777, 1760, 1740, 1715, 1685 cm^{-1} (carbonyl groups), and 1232 cm^{-1} (epoxy group). The ^{1}H and ^{13}C NMR signals (Tables 1 and 2) of **6** were assigned by different 2D NMR experiments. The combined analysis of its ^{1}H and ^{13}C NMR spectra revealed the presence of 23 carbons assigned to three methyl groups [δ_H 3.73 (δ_C 52.6), 2.06 (20.8), and 1.51 (26.3)], a trisubstituted epoxide [δ_H 4.33 (δ_C 66.6 and 59.9), an acetate on a secondary carbon [δ_H 5.41 (dd, J = 9.9, 4.5 Hz, H-13) (δ_C 67.3), 2.06 (s, H$_3$-23) (20.8)], a conjugated methylidene [δ_H 6.39 (s, H-16α) and 6.15 (s, H-16β) (δ_C 135.6 and 152.0)], four methylenes (C-2, C-7, C-9, and C-14), four methines (C-1, C-4, C-5, and C-10), an oxygen-bearing quaternary carbon (δ_C 80.1, C-8), an aldehyde [δ_H 9.45 (δ_C 193.6)], and four quaternary carbonyls [δ_C 211.7 (C-6), 169.8 (C-22), 168.8 (C-20), 167.7 (C-18)]. Detailed analysis of the 2D NMR spectra provided further structural information. The HSQC analysis revealed the assignment of each direct C−H bonding in **6** as summarized in Table 2. The skeletal structure of **6** was deduced from ^{1}H–^{1}H COSY, TOCSY, and HMBC correlations as shown in Figure 1. Interestingly, the ^{1}H and ^{13}C NMR data of bipinnatin Q (**6**), in particular the chemical shifts of the atoms ascribable to the uncommon 3-furanone moiety (C-5 through C-8 and C-19), were very similar to those reported for a series of *nor*-cembranolides isolated from a Pacific soft coral of the genus *Sinularia*.[12]

The relative stereochemistry of the eight chiral centers of bipinnatin Q (**6**) was also deduced by the analysis of NOE correlations in conjunction with $^3J_{H,H}$ analysis and extensive molecular modeling studies (Figure 2).[9] The small coupling constant between H-4 and H-5 (J = 2.9 Hz) indicated that these protons were *cis* coupled. The strong NOESY correlations of H$_3$-19/H-5, H$_3$-19/H-1, H$_3$-19/H-7β, H-5/H-4, and, most importantly, H-5/H-1 indicated that H-1, H-4, H-5, and CH$_3$-8 were all on the same side toward the β-face. As a consequence, the correlations between H-11 and H-13, H-9α and H-11, H-9α and H-10, and H-7α and H-9α indicated that H-10 and H-13 have an α-orientation. On this basis, the 11,12-epoxy and the C-13 acetate groups were assigned as having the α- and β-configuration, respectively. From a biosynthetic point of view, it is very likely that bipinnatin Q (**6**) could be obtained after further chemical modification from the known furanocembranolide bipinnatin E (**9**).[13]



The HREIMS of bipinnatin N (**7**) furnished a [M]$^+$ at *m/z* 360.1578, consistent with a molecular formula of $C_{20}H_{24}O_6$. Although the ^{13}C NMR spectrum in $CDCl_3$ displayed peaks corresponding to all 20 carbons (Table 3), many appeared as broad, low-intensity signals, suggesting that **7** contains a conformationally flexible system. That notwithstanding, the resonance lines were classified into three CH_3, four CH_2 (three sp^3 and one sp^2), six CH (four sp^3 and two sp^2), and seven quaternary carbons (two sp^3 and five sp^2) by analysis of the DEPT spectrum. The ^{1}H NMR spectrum displayed 23 proton signals (Table 3) including one epoxy proton at δ_H 3.40 (s, 1H, H-7). The IR spectrum showed the presence of hydroxyl (3392 cm^{-1}), α,β-unsaturated-γ-lactone (1749 cm^{-1}), α,β-unsaturated ketone (1685 cm^{-1}), olefin (1649 cm^{-1}), and epoxy (1275 cm^{-1}) groups. An intense UV absorption centered at λ_{max} 212 (ϵ 21 500) and carbon signal sets at δ_C 196.8 (C-3), 135.2 (C-4), 138.4 (C-5) and δ_C 175.1 (C-20), 136.7 (C-12), 146.2 (C-11) in the ^{13}C NMR spectrum confirmed the presence of two α,β-unsaturated carbonyl systems in **7**.

Comparison of the NMR data of **7** with those of the compounds already discussed revealed that bipinnatin N possesses a unique structural feature not present in compounds **1**–**6**. Specifically, the absence of NMR signals attributable to the usual C-2 and C-13 acetate groups and the presence of one oxygenated methine [δ_H 4.74 (d, 1H, J = 4.0 Hz), 76.4 (C-2)] and a likely bis-oxygenated quaternary carbon [δ_C 92.8 (C-6)] implied that an oxygen bridge is probably present between C-2 and C-6. The IR and UV spectra, in addition to the chemical shift values of the carbon atoms comprising the ensuing 6-hydroxy-4-methyl-3(2*H*)-pyranone ring system (C-2 through C-6 and C-18), supported the above deduction.[8] The gross structure of **7** was finally established by 2D NMR (including HSQC, ^{1}H–^{1}H COSY, and HMBC) analysis. The linkages of proton-bearing structural fragments with quaternary carbons and heteroatoms were fixed by HMBC spectra (Figure 1). A ketone carbonyl at δ_C 196.8 was ascribable to C-3 on the basis of its HMBC correlations with H-1, H-2, and H$_3$-18. The attachments of C-5, C-7, and C-2 (the latter through an oxa-bridge) to C-6 were established on the grounds of HMBC correlations from H-5, H-7, and H-2 to C-6. Both H-5 and H$_3$-18 correlated with C-3 and C-4 in the HMBC and indicated the presence of a $\Delta^{4(5)}$ double bond. The HMBC cross-peaks from H$_3$-17 to C-1 and from H-2 to C-1 and C-15 allowed the connection of C-1 to C-15, and those between H-11 and H$_2$-13 with C-20 (δ_C 175.1) led us to link C-12 to C-13. Finally, the linkage between C-8 and C-9 was assigned by the HMBC correlations of H$_2$-9/C-8 and H$_3$-19/C-9. The planar structure of bipinnatin N (**7**) was thus elucidated as indicated (Figure 1).

The relative stereochemistry of 3(2*H*)-pyranone **7** was demonstrated by the NOESY spectrum with the aid of proton–proton coupling constant analysis and computer-generated three-dimensional models (Figure 2).[9] The small vicinal coupling constant between H-1 and H-2 (4.0 Hz) and the NOESY correlation observed between them required that both protons be on the same β-face of the molecule. On the other hand, NOESY correlations between H$_3$-19 (δ_H 1.28) and the more shielded of the H$_2$-16 olefin protons [δ_H 4.68 (H-16β)] showed that the isopropenyl alkyl residue is on the opposite α-face as drawn. NOE correlations were also observed between the olefinic protons H-5 and H-11 and the methyl protons attached to C-8 (H$_3$-19), and H-9β had a NOE correlation to H-7. These NOE cross-peaks, in addition to those detected between H-9α/H$_3$-19, H-9αβ/H-10, H-10/H-11, H-2/H-7, and H-5/H$_3$-18 argued for the dominance of one conformation of **7** in solution. Moreover, no NOE between H-7/H$_3$-19 suggested the stereochemistry of these protons to be *trans*. From Figure 2 it is evident that the observed NOESY correlations are consistent only when the ketal hydroxy group at C-6 in **7** has the β-configuration (a distinction between the two possible diastereoisomeric structures for bipinnatin N at C-6 was achieved by a combination of NOESY and molecular

Table 3. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for Bipinnatins N and O (**7** and **8**)[a]

	bipinnatin N (**7**)		bipinnatin O (**8**)	
atom	δ_H, mult, intrgt (*J* in Hz)	δ_C (mult)[b]	δ_H, mult, intrgt (*J* in Hz)	δ_C (mult)[b]
1	3.06, 1H, brs	46.3 (CH)[c]	4.01, 1H, m	37.9 (CH)[c]
2	4.74, 1H, d (4.0)	76.4 (CH)[c]	4.83, 1H, d (4.5)	77.4 (CH)[d]
3		196.8 (C)		196.4 (C)
4		135.2 (C)		135.3 (C)
5	6.50, 1H, d (1.4)	138.4 (CH)	6.58, 1H, brs	139.0 (CH)
6		92.8 (C)		92.7 (C)
7	3.40, 1H, s	65.6 (CH)	3.45, 1H, s	65.6 (CH)
8		55.7 (C)		55.3 (C)
9α	2.31, 1H, dd (15.0, 4.0)	41.5 (CH_2)[c]	2.33, 1H, dd (15.0, 3.9)	40.6 (CH_2)[c]
9*β*	2.05, 1H, dd (15.0, 2.6)		2.09, 1H, dd (15.0, 2.5)	
10	5.12, 1H, brs	78.4 (CH)	5.19, 1H, brm	78.4 (CH)
11	6.93, 1H, s	146.2 (CH)	7.22, 1H, brd (1.4)	149.8 (CH)[c]
12		136.7 (C)		133.4 (C)[c]
13α	2.33, 1H, m	24.7 (CH_2)[d]	5.54, 1H, brs	67.5 (CH)[c]
13*β*	2.47, 1H, dd (13.9, 9.1)			
14α	2.34, 1H, m[c]	26.5 (CH_2)[d]	2.94, 1H, brs[c]	31.2 (CH_2)[d]
14*β*	2.34, 1H, m[c]		2.94, 1H, brs[c]	
15		144.2 (C)		139.1 (C)[c]
16α	4.88, 1H, brs	111.6 (CH_2)	6.34, 1H, brs	126.3 (CH_2)[c]
16*β*	4.68, 1H, brs		5.54, 1H, brs	
17	1.81, 3H, brs	23.6 (CH_3)		166.8 (C)[c]
18	1.74, 3H, d (1.4)	14.8 (CH_3)	1.72, 3H, d (1.2)	14.7 (CH_3)
19	1.28, 3H, s	21.1 (CH_3)	1.25, 3H, s	20.8 (CH_3)
20		175.1 (C)		172.1 (C)
21				170.0 (C)
22			1.99, 3H, s	21.0 (CH_3)
23			3.79, 3H, s	52.3 (CH_3)

[a] NMR spectra were recorded in $CDCl_3$ at 25 °C; ^{1}H and ^{13}C NMR chemical shift values are given in ppm and are referenced to the residual $CHCl_3$ (δ_H 7.26) or $CDCl_3$ (δ_C 77.0) signals. [b] ^{13}C NMR multiplicities were deduced from DEPT NMR experiments. [c] Low-intensity (broad) resonance. [d] Undetected carbon resonance line. The chemical shift value shown was estimated from HMBC, HSQC, and/or ^{1}H–^{1}H COSY NMR experiments.

modeling studies). The latter studies also predict that only when bipinnatin N has the 6*S** configuration (as shown in structure **7**) can the ketal hydroxy group be fixed with the lactone carbonyl oxygen in a H-bond.

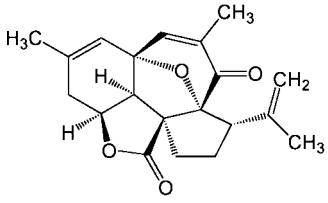
The molecular formula of bipinnatin O (**8**) was determined as $C_{23}H_{26}O_{10}$ by HREIMS. A segment of the ^{13}C NMR spectrum of bipinnatin O (Table 3), which also was characterized by the abundance of low-intensity broad resonances, was similar to that ascribed to the 6-hydroxy-4-methyl-3(2*H*)-pyranone core of bipinnatin N (**7**), implying that **8** is likely based on the same novel functionality. Two-dimensional NMR experiments, including HSQC, ^{1}H–^{1}H COSY, TOCSY, and HMBC, finally revealed the backbone of **8**, which was consistent with the above deduction. Extensive analysis of the HSQC, ^{1}H–^{1}H COSY, and HMBC spectra of **8** enabled us to outline its planar structure, which had all four ring systems identical with those of bipinnatin N (**7**) (Figure 1). The chemical shifts of both protons and carbons and the mutual HMBC correlations of CH-1, C-15, and CH_2-16 revealed the attachment of the methyl α-substituted acrylate group to C-1, and those of CH-11, C-12, CH-13, and CH_2-14 connected the sole acetate residue present in **8** to C-13. The methylcarboxylate was assigned to C-17 in view of the correlations of H_2-16/C-17 and H-1/C-17. The HMBC correlations of H-11 and H_2-14 to the oxygen-bearing C-13 (δ_C 67.5) as well as HMBC correlations of H-13 to the sp^2 carbons C-12 (δ_C 133.4) and C-21 (δ_C 170.0) confirmed the presence of an acetate group at C-13.

The NOESY spectrum of **8** showed the same relative stereochemistry as that of bipinnatin N (**7**) in all six common chiral centers (Figure 2). On the other hand, H-11 exhibited strong NOE correlations with H-10 and H-13, indicating that they were on the same side of the molecular plane. The NOESY correlations of H-1/H-2, H-2/H-7, and H-7/H-9*β* indicated that H-1, H-2, and H-7 were *β*-oriented. The NOESY spectrum also showed interactions of H_3-19 with H-9α, H-11, and H-16*β*, supporting the α-H_3C-19 assignment. The absence of NOESY correlations between H_3-19 and H-7 indicated that H_3-19 and H-7 were on opposite sides of the epoxide.

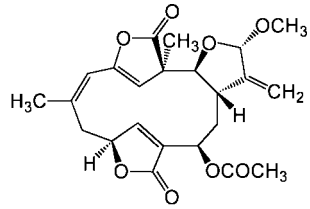
When tested for antimalarial activity against the pathogenic microbe *Plasmodium falciparum* (chloroquine-resistant clone Indochina W2), bipinnatins M (**4**), Q (**6**), and N (**7**) displayed no significant activity (IC_{50}'s > 10 *μ*g/mL). Bipinnatin M (**4**) was also inactive against the bacterium *Mycobacterium tuberculosis* (H_{37}Rv) (20% inhibition at a concentration of 128 *μ*g/mL). Of the three compounds (**4**, **6**, and **7**) submitted for evaluation to the NCI three-cell-line, one-dose, primary anticancer assay, only bipinnatin Q (**6**) showed significant cytotoxic activity. When tested against the NCI 60-cell-line cancer panel, **6** exhibited GI_{50} values of 5.8, 4.2, 4.1, and 2.6 *μ*M against the leukemia cell lines CCRF-CEM, MOLT-4, RPMI-8226, and SR, respectively. Some of the bipinnatins isolated from *Pseudopterogorgia* (**1**, **2**, **4**–**7**, and **9**) were evaluated as inhibitors of the acetylcholine-binding protein (AChBP) from *Aplysia californica*.[14] In comparing these diterpenoid analogues, a relatively small range of inhibition potencies (IC_{50}) were observed, with calculated IC_{50} values ranging from 0.23 to 0.83 *μ*M. The most potent inhibitory diterpenoid in this series lacks a C-2 acetate group and possessed both the furanyl epoxide array and the α,*β*-unsaturated aldehyde moiety in the C-1 position. Thus, bipinnatin E (**9**) had an IC_{50} value of 0.23 *μ*M at the *A. californica* nicotinic acetylcholine receptor. The present lophotoxin analogues could be useful probes for understanding the structure and function of neuronal and muscle nicotinic acetylcholine receptors, and their strong activity may also prove to be useful in a variety of clinical applications.

In recent years, a series of structurally unusual cembrane-derived polycyclic natural products have been isolated and characterized from the gorgonian coral *P. kallos*.[4] It appears quite likely that such structurally novel metabolites have their origins in the more familiar furano-*γ*-butenolide-based cembranes [i.e., bipinnatin J (**1**)], which are also found in *Pseudopterogorgia* octocorals.[15] For instance, in nearly synchronous reports, Trauner et al.[16] and Pattenden et al.[17] have independently described the biosynthetic interrelationships between bipinnatin J (**1**) and the polycyclic diterpene intricarene (**10**).[4e] Interestingly, both investigators, along

with their respective research associates, have implicated the intermediacy of a 6-hydroxy-4-methyl-3(2*H*)-pyranone, whose structure is highly reminiscent of compounds **7** and **8**. This pyranone (or its acetylated version) can undergo elimination, leading to an oxidopyrylium ion-alkene species that purportedly rearranges via a transannular 1,3-dipolar cycloaddition to yield intricarene (**10**). Thus, our discovery of bipinnatins N (**7**) and O (**8**) in *P. kallos* lends credence to their speculative biosynthetic route to **10**. Likewise, an as yet to be discovered furanocembranoid like bipinnatin K (**2**) (but having instead a $\Delta^{7(8)}$ double bond of *Z* configuration) could be the most plausible biosynthetic precursor to ciereszkolide (**11**), yet another structurally intriguing diterpenoid based on a rearranged cembrane framework.[4d] Further biomimetic studies are currently in progress in our laboratory to test the possible biosynthetic interrelationships between these highly oxidized cembranolides (and some of their ring-contracted pseudopteranolide congeners) and other natural products recently isolated from *Pseudopterogorgia* based on novel carbon architecture. The results of these studies will be reported in due course.



intricarene (**10**)



ciereszkolide (**11**)

Experimental Section

General Experimental Procedures. Optical rotations were obtained with an Autopol IV automatic polarimeter. Infrared and UV spectra were obtained with a Nicolet Magna FT-IR 750 spectrometer and Shimadzu UV-2401 PC UV–visible spectrophotometers, respectively. 1D and 2D NMR spectra were recorded with a Bruker DPX-300 or DRX-500 FT-NMR spectrometer. Mass spectrometric determinations were generated at the Mass Spectrometry Laboratory of the University of Illinois at Urbana–Champaign. The X-ray data were collected at 298 K with a Bruker SMART 1 K CCD diffractometer equipped with a graphite monochromator and Mo- Kα radiation (λ = 0.71073 Å) using the SMART software. Final values of the cell parameters were obtained from least-squares refinement. The frames were processed using the SAINT software to give the *hkl* file corrected for Lorentz and polarization effects. No absorption correction was applied. The structures were solved by direct methods with the SHELX-90 program and refined by least-squares methods on F^2, SHELXTL-93, incorporated in SHELXTL, Version 5.1. The initial E-maps yielded all non-hydrogen atom positions. All non-hydrogen atoms were refined anisotropically, and the H atoms were positioned geometrically and treated as riding, with C−H distances in the range 0.93–0.98 Å and with U_{iso}(H) = 1.2 or 1.5 U_{eq}(C). The crystallographic data for **3** and **5** reported in this article have been deposited at the Cambridge Crystallographic Data Centre, under the reference numbers CCDC 661119 and 661118, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk). Column chromatography was performed using silica gel (35–75 mesh), and TLC analysis was carried out using glass precoated silica gel plates; the spots were visualized using a UV lamp at λ = 254 nm or by exposure to I_2 vapor. HPLC was performed using either an Ultrasphere polar-bonded Cyano semipreparative column (5 μm, 10 mm × 25 cm) or an Ultrasphere normal-phase Si gel semipreparative column (5 μm, 10 mm × 25 cm). All HPLC separations were carried out using a flow rate of 2 mL/min with isocratic elution of the mobile phase and the UV detector set at λ = 220 nm. HPLC methods are described in more detail below (Method 1: NP Ultrasphere-CN column, solvent system = 85:15 hexane/2-propanol; Method 2: NP Ultrasphere-Si gel column, solvent system = 85:15 hexane/2-propanol; Method 3: NP Ultrasphere-Si gel column, solvent system = 88:12 hexane/2-propanol; Method 4: NP Ultrasphere-CN column, solvent system = 86:14 hexane/2-propanol; Method 5: NP Ultrasphere-Si gel column, solvent system = 91.5:8.5 hexane/2-propanol). All solvents used were either spectral grade or distilled from glass prior to use. The percentage yield of each compound is based on the weight of the dry gorgonian specimen.

Collection and Extraction of *Pseudopterogorgia kallos*. Fresh specimens of the sea plume *Pseudopterogorgia kallos* (phylum Cnidaria; class Anthozoa; order Gorgonacea; suborder Holaxonia; family Gorgoniidae) were collected by hand using scuba at depths of 83–91 ft off Old Providence Island, Colombia, on March 15–16, 2002. A voucher specimen (No. IPPK-01) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The organism was partially air-dried, frozen, and lyophilized prior to its extraction. The dry specimens (1.07 kg) were blended using a mixture of CH_2Cl_2/MeOH (1:1) (20 × 1 L). After filtration, the crude extract was concentrated and stored under vacuum to yield a greenish gum (166 g). The crude extract was suspended in H_2O (2L) and extracted with *n*-hexane (3 × 2 L), $CHCl_3$ (3 × 2 L), and EtOAc (2 × 2 L). Each extract was concentrated under reduced pressure to yield 71.9 g of the *n*-hexane extract, 39.3 g of the $CHCl_3$ extract, and 1.47 g of the EtOAc extract. The crude $CHCl_3$ extract, a brown, amorphous solid, was chromatographed over Si gel (673 g) using a step gradient of EtOAc/hexane as eluent and separated into 32 fractions (A−FF) on the basis of TLC and ^{1}H NMR analysis. Separation of fraction O (1.59 g) by Si gel column chromatography (75 g) in 1% acetone in $CHCl_3$ gave 13 fractions (O1−O13). Subfraction O11 (80 mg) was purified using HPLC Method 1 to yield bipinnatin N (**7**) (22.0 mg, 0.0021%). Purification of fraction P (612 mg) by size-exclusion chromatography over a Bio-Beads SX-3 column with toluene produced three subfractions, the second one (393.6 mg) of which was purified by CC over Si gel (13 g) with 15% acetone in hexane to yield 10 new subfractions. The sixth and eight subfractions were identified as bipinnatin E (**9**) and providencin[4b] (18.9 mg, 0.0018%), respectively. Further purification using HPLC Method 2 afforded pure bipinnatin E (**9**) (6.1 mg, 0.0006%).[13] Separation of fraction R (382 mg) by Si gel column chromatography (20 g) using 96:4 $CHCl_3$/EtOAc, followed by purification using HPLC Method 3, yielded 1.4 mg of bipinnatin P (**5**, 0.00013%). Bipinnatin P (**5**) was recrystallized by slow evaporation from a MeOH solution. Fraction T (510 mg) was separated in 10 subfractions (T1−T10) after Si gel column chromatography (35 g) using 2.5% of acetone in $CHCl_3$. Subfraction T4 (82.3 mg) was purified using HPLC Method 1 to afford more bipinnatin P (**5**) (1.3 mg, 0.000028%) and bipinnatin Q (**6**) (14.2 mg, 0.0013%). Fraction U (849 mg) was separated into 12 fractions (U1−U12) by Si gel column chromatography using 95:5 $CHCl_3$/EtOAc as mobile phase. Subsequent purification of subfraction U5 (90.6 mg) using HPLC Method 1 afforded bipinnatin K (**2**) (7.5 mg, 0.0007%) and more bipinnatin Q (**6**) (12.9 mg, 0.0012%). Fraction V (1.23 g) was separated over Si gel (65 g) using 97:3 $CHCl_3$/EtOAc to give 18 subfractions (V1−V18). Subfractions V7 (33.2 mg), V8 (48.1 mg), and V9 (78.1 mg) were combined and purified using HPLC Method 5 to afford bipinnatin O (**8**) (27.8 mg, 0.0026%). Fraction Z (883 mg) was separated into 13 subfractions (Z1−Z13) using column chromatography over Si gel (47 g) with 20% acetone in $CHCl_3$. Subfraction Z10 (140.9 mg, 0.013%) was identified as bipinnatin M (**4**). Further purification of subfraction Z12 (32.6 mg) using HPLC Method 4 afforded 4.4 mg of bipinnatin L (**3**, 0.00041%). Bipinnatin L was recrystallized by slow evaporation from a EtOAc solution.

Bipinnatin K (2): colorless solid; $[\alpha]_D^{20}$ –31.5 (*c* 0.7, $CHCl_3$); IR (neat) ν_{max} 3510, 3097, 3022, 2934, 1761, 1736, 1717, 1447, 1374, 1235, 1136, 1102, 1032, 962, 757 cm^{-1}; UV (MeOH) λ_{max} 209 (ϵ 4300) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) (see Table 1) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); EIMS *m*/*z* $[M]^+$ 446 (2), 403 (1), 388 (4), 386 (4), 354 (4), 329 (2), 221 (100), 153 (13), 125 (28); HRFABMS (glycerol) *m*/*z* $[M + 1]^+$ 447.1660 (calcd for $C_{23}H_{27}O_9$, 447.1655).

Bipinnatin L (3): yellowish oil; $[\alpha]_D^{20}$ +78.7 (*c* 0.75, MeOH); IR (neat) ν_{max} 3435, 3094, 3019, 2951, 1750, 1717, 1627, 1439, 1373, 1238, 1150, 1090, 755 cm^{-1}; UV (MeOH) λ_{max} 207 (ϵ 9400), 267 (ϵ 600) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) (see Table 1) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); HRESIMS *m*/*z* $[M + Na]^+$ 561.1561 (calcd for $C_{25}H_{30}O_{13}Na$, 561.1584).

Single-Crystal X-Ray Structure Determination of Bipinnatin L (3) at 298(2) K. Compound **3** was recrystallized by slow evaporation from EtOAc. Crystal Data: $C_{25}H_{30}O_{13}\cdot H_2O$, M_r = 556.51, orthorhombic, space group $P2_12_12_1$, a = 8.947(5) Å, b = 10.719(4) Å, c = 27.826(10) Å, V = 2669(2) Å^3, Z = 4, ρ_{calc} = 1.385 Mg m^{-3}, F_{000} = 1176, λ(Mo Kα) = 0.71073 Å, μ = 0.114 mm^{-1}. Data collection and reduction:

crystal size, 0.12 × 0.11 × 0.02 mm, θ range = 1.46–28.36°, 20 340 reflections collected, 6381 independent reflections (R_{int} = 0.1117), final R indices ($I > 2\sigma(I)$): R_1 = 0.0624, wR_2 = 0.1124 for 359 variable parameters, GOF = 0.933.

Bipinnatin M (4): colorless, crystalline solid; $[\alpha]_D^{20}$ +91.7 (*c* 1.2, MeOH); IR (neat) ν_{max} 3534, 3388, 3276, 3099, 2949, 1754, 1742, 1728, 1719, 1444, 1371, 1239, 1137, 1087, 969, 909, 789 cm^{-1}; UV (MeOH) λ_{max} 202 (ϵ 17 800) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) (see Table 1) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); HRESIMS *m/z* [M + Na]$^+$ 545.1631 (calcd for $C_{25}H_{30}O_{12}Na$, 545.1635).

Bipinnatin P (5): colorless, crystalline solid; $[\alpha]_D^{20}$ +90.0 (*c* 1.0, MeOH); IR (neat) ν_{max} 3080, 3021, 2954, 2855, 1767, 1714, 1678, 1625, 1601, 1439, 1372, 1235, 1152, 1097, 1030, 959, 755 cm^{-1}; UV (MeOH) λ_{max} 202 (ϵ 11 900), λ_{max} 247 (ϵ 4800) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) (see Table 1) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); HRESIMS *m/z* [M + Na]$^+$ 527.1521 (calcd for $C_{25}H_{28}O_{11}Na$, 527.1529).

Single-Crystal X-Ray Structure Determination of Bipinnatin P (5) at 298(2) K. Compound **5** was recrystallized by slow evaporation from MeOH. Crystal Data: $C_{25}H_{28}O_{11}$, M_r = 504.47, monoclinic, space group $P2_1$, a = 10.028(3) Å, b = 11.478(3) Å, c = 11.194(4) Å, V = 1262.5(6) Å^3, Z = 2, ρ_{calc} = 1.327 Mg m^{-3}, F_{000} = 532, λ(Mo Kα) = 0.71073 Å, μ = 0.105 mm^{-1}. Data collection and reduction: crystal size, 0.15 × 0.12 × 0.10 mm, θ range = 1.86–28.23°, 8360 reflections collected, 4766 independent reflections (R_{int} = 0.0430), final R indices ($I > 2\sigma(I)$): R_1 = 0.0512, wR_2 = 0.0947 for 330 variable parameters, GOF = 1.047.

Bipinnatin Q (6): yellowish oil; $[\alpha]_D^{20}$ +15.0 (*c* 1.0, $CDCl_3$); IR (neat) ν_{max} 3017, 2953, 2933, 2852, 2707, 1777, 1760, 1740, 1715, 1685, 1436, 1370, 1232, 1113, 1079, 1028, 962, 902, 753 cm^{-1}; UV (MeOH) λ_{max} 213 (ϵ 6700), λ_{max} 244 (ϵ 1700) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) (see Table 1) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); EIMS *m/z* [M]$^+$ 478 (4), 447 (32), 446 (100), 418 (13), 94 (11), 92 (36), 79 (13), 77 (45); HRESIMS *m/z* [M + Na]$^+$ 501.1368 (calcd for $C_{23}H_{26}O_{11}Na$, 501.1373).

Bipinnatin N (7): yellowish oil; $[\alpha]_D^{20}$ –138.0 (*c* 1.0, MeOH); IR (neat) ν_{max} 3392, 3088, 3013, 2931, 2857, 1749, 1685, 1649, 1448, 1379, 1275, 1157, 1084, 1030, 895, 756 cm^{-1}; UV (MeOH) λ_{max} 212 (ϵ 21 500) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 3); EIMS *m/z* [M]$^+$ 360 (7), 342 (25), 327 (9), 290 (13), 194 (29), 167 (96), 153 (77), 137 (52), 111 (93), 97 (50), 69 (100); HREIMS *m/z* [M]$^+$ 360.1578 (calcd for $C_{20}H_{24}O_6$, 360.1573).

Bipinnatin O (8): yellowish oil; $[\alpha]_D^{20}$ +79.6 (*c* 0.7, MeOH); IR (neat) ν_{max} 3412, 3083, 3022, 2952, 2926, 2857, 1760, 1736, 1716, 1690, 1647, 1627, 1442, 1372, 1235, 1158, 1093, 1030, 1016, 966, 826, 762 cm^{-1}; UV (MeOH) λ_{max} 205 (ϵ 35 400) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 3); EIMS *m/z* [M]$^+$ 462 (1), 444 (9), 431 (7), 402 (9), 385 (22), 384 (17), 300 (19), 218 (26), 204 (44), 167 (95), 153 (100), 137 (88), 126 (75), 111 (91), 91 (56), 69 (65); HREIMS *m/z* [M]$^+$ 462.1525 (calcd for $C_{23}H_{26}O_{10}$, 462.1526).

Bipinnatin E (9): amorphous, colorless solid; $[\alpha]_D^{20}$ –17.5 (*c* 1.26, $CHCl_3$); IR (neat) ν_{max} 3133, 3023, 2955, 2853, 2708, 1783, 1745, 1731, 1714, 1690, 1621, 1580, 1443, 1376, 1231, 1079, 976, 759 cm^{-1}; UV (MeOH) λ_{max} 215 (ϵ 19 200), λ_{max} 247 (ϵ 8500) nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.41 (1H, brm, H-1), 3.25 (1H, dd, J = 12.2, 18.8 Hz, H-2α), 2.95 (1H, dd, J = 3.1, 18.7 Hz, H-2β), 6.54 (1H, brd, J = 1.2 Hz, H-5), 4.08 (1H, brs, H-7), 2.50 (1H, dd, J = 2.8, 15.4 Hz, H-9α), 2.08 (1H, dd, J = 4.4, 15.4 Hz, H-9β), 4.79 (1H, dd, J = 2.7, 4.4 Hz, H-10), 4.17 (1H, s, H-11), 4.99 (1H, brd, J = 6.4 Hz, H-13), 2.73 (1H, ddd, J = 7.5, 10.8, 15.8 Hz, H-14α), 1.86 (1H, brd, J = 15.6 Hz, H-14β), 6.47 (1H, brs, H-16α), 6.11 (1H, brs, H-16β), 9.68 (1H, s, H-17), 1.14 (3H, s, H-19), 3.78 (3H, s, H-21), 1.97 (3H, s, H-23); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 30.6 (CH, br, C-1), 33.4 (CH_2, C-2), 158.6 (C, C-3), 114.2 (C, C-4), 108.0 (CH, C-5), 148.4 (C, C-6), 55.4 (CH, C-7), 55.9 (C, C-8), 39.1 (CH_2, C-9), 76.6 (CH, C-10), 64.0 (CH, C-11), 61.1 (C, C-12), 70.2 (CH, C-13), 31.2 (CH_2, C-14), 152.9 (C, C-15), 134.5 (CH_2, br, C-16), 193.3 (CH, C-17), 163.7 (C, C-18), 20.3 (CH_3, C-19), 167.8 (C, C-20), 51.5 (CH_3, C-21), 169.8 (C, C-22), 20.5 (CH_3, C-23); EIMS *m/z* [M]$^+$ 460 (25), 429 (44), 400 (70), 368 (43), 340 (24), 318 (35), 261 (27), 236 (42), 219 (51), 168 (99), 165 (66), 137 (100); HREIMS *m/z* [M]$^+$ 460.1371 (calcd for $C_{23}H_{24}O_{10}$, 460.1369).

Attempted Epoxidation of Bipinnatin M (4). A mixture of bipinnatin M (**4**) (6.1 mg, 0.01 mmol), *m*-CPBA (3.1 mg, 0.02 mmol), and Na_2HPO_4 (2.1 mg, 0.01 mmol), dissolved in CH_2Cl_2 (5 mL), was stirred at 25 °C overnight. Thereafter, the reaction mixture was heated at 35 °C for 5 h. Routine TLC analysis revealed that no reaction took place. An attempt to oxidize **4** using magnesium monoperoxyphtalate (MMPP) in methanol (first at 25 °C for 48 h and then at 65 °C for 24 h) failed to give any reaction products.

Attempted Acid-Catalyzed Dehydration of Bipinnatin M (4). To a solution of bipinnatin M (**4**, 26.1 mg, 0.05 mmol) in 3:1 THF/H_2O (8 mL) was added a small crystal of PTSA·H_2O. After stirring at 25 °C for 5 h, a saturated solution of $NaHCO_3$ (10 mL) was added and the resulting mixture was extracted with $CHCl_3$ (3 × 7 mL). The combined organic layer was concentrated, stored under vacuum, and analyzed by TLC and NMR to yield an intractable mixture of products.

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Supporting Information Available: The molecular structures of some reference compounds, namely, bipinnatins A–I (*P. bipinnata*), lophotoxin (*Lophogorgia rigida*), providencin, bielshowskysin, and kallosin A (*P. kallos*). This information is available free of charge via the Internet at http://pubs.acs.org.

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(5) (a) Due to the close structural relatedness to metabolites previously isolated from the gorgonian species *Pseudopterogorgia bipinnata*, the trivial names assigned to compounds **2–8** are based on the previous work by Fenical et al. and Wright et al., see: Culver, P.; Burch, M.; Potenza, C.; Wasserman, L.; Fenical, W.; Taylor, P. *Mol. Pharmacol.* **1985**, *28*, 436–444. (b) Fenical, W. *J. Nat. Prod.* **1987**, *50*, 1001–1008. (c) Wright, A. E.; Burres, N. S.; Schulte, G. K. *Tetrahedron Lett.* **1989**, *30*, 3491–3494.
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(8) As far as we have been able to ascertain, this is the first report of a stable natural product possessing this unprecedented structural motif.
(9) Lowest energy conformers were searched using MMFF force field implemented in the McSpartan '04 Program (Wavefunction, Inc.).
(10) Bipinnatin M (**4**) is a likely photooxidation product of the lophotoxin analogue bipinnatin I, a known furanocembranolide first isolated from an unidentified *Pseudopterogorgia* species and subsequently from *P. bipinnata*; see: Abramson, S. N.; Trishman, J. A.; Tapiolas, D. M.; Harold, E. E.; Fenical, W.; Taylor, P. *J. Med. Chem.* **1991**, *34*, 1798–1804, and references therein.[7]
(11) Interestingly, while diterpenes **3** and **4** contain similarly functionalized chiral groups with identical relative configuration (i.e., C-1 through C-8), significant differences in the chemical shifts of some of the atoms at (or near) these positions were observed. On the basis of our NMR and molecular modeling data, it appears that most of these deviations

(other than those due to the presence in **4** of a $\Delta^{4(5)}$ olefin) can be attributed to differences in the solution conformations for **3** and **4** and not to changes in the relative stereochemistry of **4**.
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