New Metabolites from the West Indian Sea Feather *Pseudopterogorgia bipinnata*

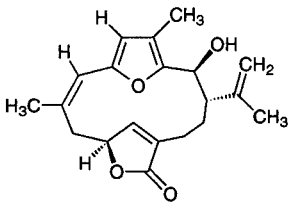
Abimael D. Rodríguez* and Yan-Ping Shi

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, San Juan, Puerto Rico 00931-3346, and Center for Molecular and Behavioral Neuroscience, Universidad Central del Caribe, Bayamón, Puerto Rico 00960-3001

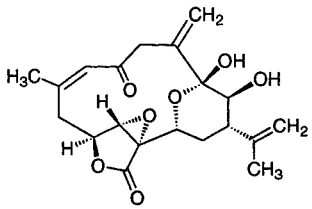
Received May 5, 2000

Two diterpenes, a novel *seco*-furanocembranolide (**3**) and the highly oxygenated cembranolide bipinnatolide K (**4**), have been isolated from the West Indian gorgonian octocoral *Pseudopterogorgia bipinnata*. The chemical structures of these compounds were determined by 1D and 2D NMR spectroscopy in combination with IR, UV, and HRFABMS analyses.

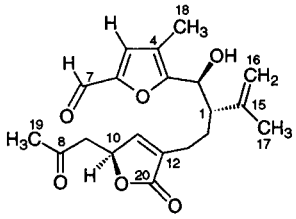
Caribbean gorgonian octocorals of the genus *Pseudopterogorgia* are prolific producers of metabolites derived from the mevalonic acid pathway.[1] Metabolites produced from geranylgeranyl pyrophosphate are especially plentiful and include the pseudopteranes, serrulatanes, amphilectanes, elisabethanes, and cembranes.[1,2] The bipinnatins (i.e., **1**) and the bipinnatolides (i.e., **2**), isolated from *Pseudopterogorgia bipinnata* (Verrill, 1864) (order Gorgonacea, family Gorgoniidae, phylum Cnideria), are examples of highly functionalized members of the latter group of diterpenes.[3,4] We now wish to report the isolation and structure elucidation of *seco*-bipinnatin J (**3**) and bipinnatolide K (**4**) from a specimen of *P. bipinnata* collected in 1996 near San Andrés Island, Colombia.



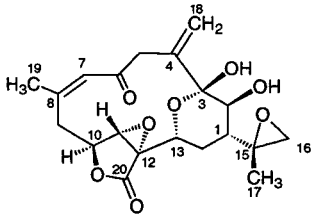
bipinnatin J (1)



bipinnatolide G (2)



***seco*-bipinnatin J (3)**



bipinnatolide K (4)

Dry samples of *P. bipinnata* were homogenized and extracted with MeOH−$CHCl_3$ (1:1) to give a crude extract. This extract was partitioned between hexane, $CHCl_3$, and *n*-butanol. The $CHCl_3$-soluble material was further fractionated by column chromatography using Si gel (step gradient of EtOAc−hexane), resulting in the separation of a number of highly colored bands, including yellow, red, orange, green, and black. Samples were inspected by TLC and NMR spectroscopy and purified by repetitive size-exclusion chromatography using Bio-Beads SX-3 (toluene) and Si gel column chromatography to give *seco*-bipinnatin J (**3**) and bipinnatolide K (**4**) as colorless materials.

seco-Bipinnatin J (**3**) was obtained as a colorless gum that yielded an intense FABMS [M + Na] ion at *m/z* 383 corresponding to the molecular formula $C_{20}H_{24}O_6$ (HR-FABMS *m/z* $[M + Na]^+$ 383.1470). Its IR spectrum suggested the presence of hydroxyl (3468 cm^{-1}), α,*β*-unsaturated ester (1753 cm^{-1}), α,*β*-unsaturated aldehyde (1671 cm^{-1}), and ketone (1718 cm^{-1}) groups. NMR experiments were performed in $CDCl_3$ to enhance peak separation; however, peak broadening in the ^1H NMR as well as peak splitting in the ^{13}C NMR spectra was observed, suggesting the presence of at least two distinct conformations in solution. The ^1H NMR spectrum of **3** showed a sharp one-proton singlet at *δ* 9.52, indicating the presence of a conjugated aldehyde, two broad singlets at *δ* 5.10 and 4.99 (1H each) with allylic couplings to a broad methyl singlet at *δ* 1.74, suggesting the presence of an isopropylene group, and a sharp methyl singlet at *δ* 2.20 typical of a CH_3CO group. Other features of the spectrum included a broad one-proton singlet at *δ* 7.06 and a broad methyl singlet at *δ* 2.11, indicative of a *β*-methylfuran moiety, a one-proton singlet at *δ* 7.03, indicating an α,*β*-unsaturated *γ*-lactone, a one-proton doublet of doublet at *δ* 5.24 ($J = 5.7$, 6.6 Hz), suggesting a hydrogen atom on the carbon bearing the lactone oxygen, and a one-proton doublet at *δ* 4.58 ($J =$ 9.9 Hz) ascribable to a hydrogen on a carbon bearing an allylic hydroxyl group.[3,4] The ^{13}C NMR spectrum exhibited 20 signals ($3CH_3$, $4CH_2$, 6CH, and 7C) whose chemical shift values and multiplicities (DEPT) confirmed the presence of a 4-methylfuran-2-carbaldehyde [*δ* 177.5 (d), 156.1 (s), 151.0 (s), 124.7 (d), 121.1 (s), 9.6 (q)], an α,*γ*-disubstituted butenolide [*δ* 172.9 (s), 148.0 (d), 133.6 (s), 76.8 (d)], an isopropylene group [*δ* 142.7 (s), 117.6 (t), 17.6 (q)], a hydroxyl-bearing carbon [*δ* 66.6 (d)], and a nonconjugated ketone carbonyl bearing a methyl group [*δ* 204.4 (s), 30.4 (q)]. From these spectral data we deduced that compound **3** was bicyclic with four olefins and three carbonyl groups. The ^1H NMR chemical shifts of **3** (Table 1) were very similar to those of bipinnatin J (**1**)[5] with the exception that the resonances associated with the trisubstituted Δ^7 olefin of the latter [*δ* 6.09 (br s, 1H, H-7) and 1.98 (br s, 3H, Me-19)] were replaced by a methyl singlet at *δ* 2.20 and a sharp one-proton singlet at *δ* 9.52. That cleavage of the Δ^7 olefin group of **1** had indeed occurred to yield compound **3** was further confirmed by the ^{13}C NMR spectrum, which showed signals at *δ* 177.5 (d) and 30.4 (q) corresponding to the signals at *δ* 9.52 (br s, 1H) and 2.20 (s, 3H) in the ^1H

* To whom correspondence should be addressed. Tel.: (787)-764-0000, ext 4799. Fax: (787)-751-0625. E-mail: arodrig@goliath.cnnet.clu.edu.

10.1021/np000224v CCC: $19.00
Published on Web 09/21/2000

Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, and HMBC Spectral Data for Compounds **3** and **4**[a]

	seco-bipinnatin J (**3**)[b]				bipinnatolide K (**4**)[c]			
atom	δ_H, mult, intgr (*J* in Hz)	δ_C (mult)	COSY	HMBC[d]	δ_H, mult, intgr (*J*)	δ_C (mult)	COSY	HMBC[d]
1	2.72, m, 1H	52.1 (d)	H2, H14α*β*	H16α*β*, H17	1.74, ddd, 1H (3.3, 10.5, 12.6)	42.0 (d)	H2, H14α*β*	H14α, H17
2	4.58, br d, 1H (9.9)	66.6 (d)	H1	H1	3.38, br d, 1H (10.5)	69.1 (d)	H1	H14α*β*
3		151.0 (s)		H5		97.4 (s)		H5*β*, H18α*β*
4		121.1 (s)		H18		142.6 (s)		H5α*β*
5α	7.06, br s, 1H	124.7 (d)	H18	H18	3.07, d, 1H (15.3)	50.8 (t)	H5*β*, H7, H18α*β*	H18α*β*
5*β*					3.19, d, 1H (15.3)		H5α	
6		156.1 (s)		H5, H18		198.7 (s)		H5α*β*, H7
7	9.52, s, 1H	177.5 (d)		H5	6.19, br s, 1H	126.1 (d)	H5α, H9*β*, H19	H5α, H9α*β*, H19
8		204.4 (s)		H9α*β*, H19		147.8 (s)		H9α*β*, H10, H19
9α	2.95, dt, 1H (5.7, 17.4)	46.3 (t)	H9*β*, H10	H10, H19	2.49, br dd, 1H (7.5, 12.9)	33.0 (t)	H9*β*, H10	H7, H19
9*β*	2.65, dt, 1H (6.6, 17.4)		H9α, H10		4.08, br d, 1H (12.9)		H7, H9α	
10	5.24, br dd, 1H (5.7, 6.6)	76.8 (d)	H9α*β*, H11	H9α*β*, H11	4.86, br d, 1H (7.5)	78.2 (d)	H9α	H9α, H11
11	7.03, br s, 1H	148.0 (d)	H10	H9α*β*	4.20, br s, 1H	58.5 (d)		H9α*β*, H10
12		133.6 (s)		H13		58.9 (s)		H10, H13, H14α
13	2.15, m, 2H	22.8 (t)	H14α*β*	H1, H11	4.62, dd, 1H (3.0, 12.3)	62.6 (d)	H14α*β*	H14α
14α	1.18, m, 1H	25.7 (t)	H1, H13	H1, H13	0.81, ddd, 1H (12.3, 12.3, 12.6)	27.4 (t)	H1, H13, H14*β*	
14*β*	1.52, m, 1H				1.53, ddd, 1H (3.0, 3.3, 13.2)		H1, H13, H14α	
15		142.7 (s)		H1, H17		57.8 (s)		H16α*β*, H17
16α	5.10, br s, 1H	117.6 (t)	H16*β*, H17	H1, H17	2.52, br d, 1H (3.9)	52.0 (t)	H16*β*	H17
16*β*	4.99, br s, 1H		H16α, H17		2.59, br d, 1H (3.9)		H16α, H17	
17	1.74, br s, 3H	17.6 (q)	H16α*β*	H1, H16α*β*	1.28, br s, 3H	17.9 (q)	H16*β*	H16α
18α	2.11, br s, 3H	9.6 (q)	H5	H5	5.52, br s, 1H	119.0 (t)	H5α, H18*β*	H5α*β*
18*β*					5.25, br s, 1H		H5α, H18α	
19	2.20, s, 3H	30.4 (q)			1.83, br s, 3H	28.9 (q)	H7	H7, H9α*β*
20		172.9 (s)		H11		171.5 (s)		H10

[a] Assignments were aided by ^{1}H−^{1}H COSY, spin splitting patterns, DEPT, HMBC, HMQC, and NOESY experiments 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 $CDCl_3$. [c] Data recorded in a mixture of $CDCl_3$ and CD_3OD. [d] Protons correlated to carbon resonances in atom column. Parameters were optimized for $^{2,3}J_{CH} = 6$ and 8 Hz.

NMR spectrum, respectively (HMQC). The large ^{1}H NMR coupling constant of the oxymethine proton H-2 resonating at δ 4.58 (d, 1H, $J = 9.9$ Hz) indicated a *trans* relationship to H-1. A weak but very diagnostic NOE between H-2 and the vinyl methyl protons at C-15 confirmed the *cis* relative stereochemistry between H-2 and the alkenyl chain at C-1. The relative configuration of the lactone proton at C-10 was assigned to be on the same side as the H-2 proton by comparison of the ^{1}H NMR chemical shift, multiplicity, and coupling constant spectral data for H-11 with those of bipinnatin J (**1**) [δ 7.03 (br s, $^3J_{H10-H11} \leq 1$ Hz) in **3** and δ 6.83 (br s, $^3J_{H10-H11} \leq 1$ Hz) in **1**].[5] The structure of **3** was further supported by 2D NMR spectra including COSY, NOESY, HMQC, and HMBC (Table 1). Furanocembranolide bipinnatin J (**1**) is a logical precursor to **3** upon oxidative cleavage of its Δ^7 olefin function. Since the absolute configuration of bipinnatin J has been established (its structure has been correlated chemically with that of furanopseudopterane kallolide A),[5,6] we have assumed that the *seco* derivative **3** also has the same absolute configuration at all common chiral centers.

Bipinnatolide K (**4**) was isolated as colorless crystals, $[\alpha]^{24}_D$ −9.2°, with the formula $C_{20}H_{24}O_8$ established by HRFABMS, and contained one oxygen atom more than in the molecular formula of bipinnatolide G (**2**).[4] Like **2**, compound **4** showed IR absorptions (3447, 1772, 1669, and 1247 cm^{-1}) that indicated the presence of hydroxyl, ester, conjugated ketone, and epoxide functionalities, respectively. That many of the same structural features found in **2** were also present in bipinnatolide K (**4**) was supported by a UV absorption at $\lambda_{max} = 210$ nm (ϵ 62 400). Comparison of the ^{1}H and ^{13}C NMR spectra of **4** (Table 1) with those of **2** confirmed the structural similarity of these compounds and revealed the presence of a feature unique to **4**. The α,*β*-epoxy *γ*-lactone [δ 171.5 (s), 78.2 (d), 58.9 (s), and 58.5 (d)], the *β*-methyl α,*β*-unsaturated enone [δ 198.7 (s), 147.8 (s), 126.1 (d), and 28.9 (q)], the cyclic hemiacetal [δ 97.4 (s)], the terminal olefin [δ 142.6 (s) and 119.0 (t)], and the secondary hydroxyl group [δ 69.1 (d)] were assumed to be intact in **4** on the basis of similar IR, UV, and NMR data. Bipinnatolide K (**4**), however, had one oxygen-bearing methylene, as indicated by a ^{13}C NMR signal at δ 52.0 (t) and ^{1}H signals at δ 2.59 (br d, 1H, $J = 3.9$ Hz) and 2.52 (br d, 1H, $J = 3.9$ Hz). Also, resonances at δ 58.9 (s) and 57.8 (s) in the ^{13}C NMR spectrum confirmed the presence in this compound of two epoxy-bearing quaternary carbons, a feature not found in **2**. Clearly, bipinnatolide K, like the known furanocembranolide bipinnatin C,[3] contained an additional epoxy group at C-15,16 rather than a terminal olefin, as in metabolite **2**. This would accommodate the nine degrees of unsaturation and the number of oxygen atoms required by the molecular formula of **4**. A 2D ^{1}H NMR homonuclear correlation experiment (COSY, see Table 1) allowed the assignment of all the signals in the ^{1}H NMR spectrum of **4**. Chemical shifts of the protonated carbons were assigned by 2D ^{1}H−^{13}C heterocorrelation experiments (HMQC and HMBC). A NOESY experiment showed that H-1 and H-13, H-2 and Me-17, H-9α and H-10, H-13 and H-14*β*, H-11 and H-14α, H-7 and Me-19, H-2 and H-5α, and H5*β* and H-18*β* were within NOE proximity, which confirmed the relative stereochemistry depicted in **4**. This compound has a logical structure from a biosynthetic viewpoint. Thus, bipinnatolide G (**2**) could be envisioned as a precursor for bipinnatolide K via oxidation, in this case at C-15,16.

In an in vitro antituberculosis screen against *Mycobacterium tuberculosis* $H_{37}Rv$ at 6.25 *μ*g/mL *seco*-bipinnatin J (**3**) and bipinnatolide K (**4**) caused no inhibition.

Experimental Section

General Experimental Procedures. Infrared spectra were recorded with a Nicolet Magna FT-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, NOESY, DEPT, HMQC, and HMBC experiments were mea-

sured with a General Electric QE-300 spectrometer. Column chromatography was performed on Si gel (35−75 mesh). TLC analyses were carried out using Analtech glass-packed precoated Si gel plates. 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 crude gorgonian extract.

Animal Material. Bipinnatin J (**1**), bipinnatolide G (**2**), *seco*-bipinnatin J (**3**), and bipinnatolide K (**4**) were isolated from the gorgonian octocoral *Pseudopterogorgia bipinnata* (2.1 kg) collected in May, 1996, near San Andrés Island, Colombia. A voucher specimen of *P. bipinnata* (no. PBSAI-01) is stored at the Chemistry Department of the University of Puerto Rico.

Extraction and Isolation. The extraction scheme and isolation of metabolites **1** and **2** have been previously described.[4,5] The $CHCl_3$ extract (43.3 g) was chromatographed over Si gel (400 g). Fractions were pooled based on their TLC and NMR profile to yield 30 primary fractions, designated as I−XXX. Fraction XVII (1.4 g) was further purified by size-exclusion chromatography (Bio-Beads SX-3) using toluene as eluant. Four tertiary fractions were obtained (XVIIA−XVIID). Fraction D (0.8 g) was purified by column chromatography over Si gel (15 g) using a 20:1 mixture of $CHCl_3$ in acetone to yield *seco*-bipinnatin J (**3**) (10.5 mg; 6.25×10^{-3}% yield) and bipinnatolide K (**4**) (4.0 mg; 2.38×10^{-3}% yield).

***seco*-Bipinnatin J (3):** colorless gum; $[\alpha]^{24}_D$ +16.3° (*c* 0.86, $CHCl_3$); UV (MeOH) λ_{max} 206 (ϵ 14 700) and 292 nm (ϵ 10 000); IR (film) 3468, 3078, 1753, 1718, 1671, 1627, 1525, 1370, 1068, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HRFAB-MS (3-NBA) *m*/*z* $[M + Na]^+$ 383.1470 (calcd for $C_{20}H_{24}O_6Na$, 383.1471).

Bipinnatolide K (4): colorless crystals; mp 199−200 °C dec; $[\alpha]^{24}_D$ −9.2° (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} 210 nm (ϵ 62 400); IR (film) 3447, 2959, 2924, 1772, 1669, 1655, 1247, 1096, 1047 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD, 300 MHz) and ^{13}C NMR ($CDCl_3$ + CD_3OD, 75 MHz) (see Table 1); HRFAB-MS (3-NBA) *m*/*z* $[M + Na]^+$ 415.1351 (calcd for $C_{20}H_{24}O_8Na$, 415.1368).

Acknowledgment. This report is contribution No. 7 of the CMBN (Center for Molecular and Behavioral Neuroscience). The CMBN program is sponsored in part by grants NCRR RCMI-2G12RR03035 and NIGMS RO1-GM52277. HRFAB spectral determinations were performed by the Midwest Center for Mass Spectrometry, a NSF Regional Facility. Ms. Janet Figueroa obtained the IR spectra from the Material Characterization Center (MCC) at UPR. We are indebted to Dr. Robert C. Reynolds (TAACF) for in vitro evaluation of antituberculosis activity. Support for this research at UPR was kindly provided by the NSF-EPSCoR, NIH-MBRS, and NSF-MRCE programs.

References and Notes

(1) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618, and references therein.
(2) Faulkner, D. J. *Nat. Prod. Rep*. **2000**, *17*, 7−55, and previous reports in this series.
(3) Wright, A. E.; Burres, N. S.; Schulte, G. K. *Tetrahedron Lett*. **1989**, *30*, 3491−3494.
(4) Rodríguez, A. D.; Shi, J.-G.; Huang, S. D. *J. Nat. Prod*. **1999**, *62*, 1228−1237.
(5) Rodríguez, A. D.; Shi, J.-G. *J. Org. Chem*. **1998**, *63*, 420−421.
(6) (a) Marshall, J. A.; Bartley, G. S.; Wallace, E. M. *J. Org. Chem*. **1996**, *61*, 5729−5735. (b) Marshall, J. A.; Liao, J. *J. Org. Chem*. **1998**, *63*, 5962−5970.

NP000224V