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CYTOTOXIC CEMBRANOIDS FROM THE GORGONIAN *PSEUDOPTEROGORGIA BIPINNATA*

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Abstract: Four new cytotoxic cembranoids, denoted as bipinnatins a-d, have been isolated from the gorgonian coral *Pseudopterogorgia bipinnata*. Their structures were determined through a combination of spectroscopic and x-ray crystallographic methods.

Until recently, few cembranoids had been reported from the gorgonian soft coral genus *Pseudopterogorgia*. In a recent paper, Fenical reported the isolation of two cembranoids with potent antiinflammatory activity from the sea whip *Pseudopterogorgia bipinnata*[1]. As part of our investigations of new antitumor agents derived from marine sources, we would like to report the isolation of four new, related cembranoids from *P. bipinnata*, **1-4**, which we have called bipinnatins a-d, respectively[2]. Three of these compounds exhibit potent cytotoxic activity.

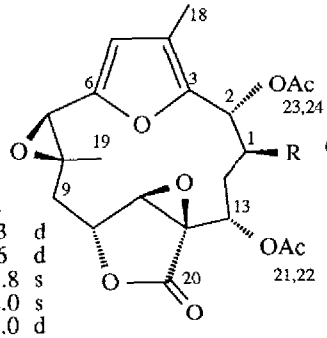
Crude shipboard extracts of *P. bipinnata* collected off Jamaica Cay, Acklins Island, Bahamas in July of 1987 exhibited *in vitro* inhibition of P388 murine tumor cell replication[3]. Bioassay-guided purification led to the isolation of four compounds, the structures of which were determined through the interpretation of the nmr, ir, mass spectral and x-ray crystallographic data. An ethyl acetate extract prepared from 37 g (wet weight) of the frozen gorgonian was chromatographed under vacuum liquid chromatographic conditions on a silica gel stationary phase using a step gradient of ethyl acetate-heptane as eluent. Active fractions were purified further by HPLC utilizing either isopropanol-heptane (1:3) on a CYANO column (EM Science) followed by ethyl acetate-heptane (1:1) on a silica gel column (Whatman Partisil) or the CYANO column alone to yield pure bipinnatins a (28 mg), b (18.7 mg), c (16mg) and d (2.4 mg).

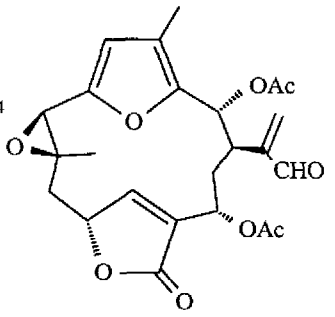
Initial inspection of the proton and carbon nmr spectra of **1-4** (Tables 1, 2), suggested that the structures of a-d are closely related. The proton and carbon nmr spectra of **1**, **2** and **4** contain a number of broadened resonances which led to low sensitivity in 2-D hetero correlated nmr experiments. However, the nmr spectra of compound **3** contained only sharp resonances and therefore, the majority of the structural characterization was carried out on **3**. Mass spectral analysis of **3** confirmed a formula of $C_{24}H_{28}O_{10}$ (HREI m/z_{obs}=476.1688; m/z_{calc}=476.1683) which requires eleven sites of unsaturation. The carbon nmr spectra suggested the presence of three carbonyl groups. A trisubstituted furan, as found in kallolide a[4], was suggested by the proton nmr resonance observed at 5.97 (s) ppm and the carbon resonances observed at 145.9 (s), 121.3 (s), 109.8 (d) and 149.4 (s). Long range proton-carbon correlations between the proton observed at 5.97 ppm and the carbons observed at 149.4 and 145.9 ppm lent further support to this assignment. As no further unsaturation was evident from the nmr spectra, **3** must contain six rings. Analysis of a 2D-HMQC[5] experiment in which both the one bond carbon-hydrogen correlations and coupling constants are observed, suggested that a number of small rings such as epoxides were present in **3** (δ: 64.9 (d $^1J_{CH}$=195 Hz); 56.2 (d $^1J_{CH}$=180 Hz); 50.7 (t $^1J_{CH}$=168 Hz)). An unusual ir absorption at 1781 cm^{-1} suggested the presence of an α,β-epoxy lactone as found in lophotoxin[6]. The presence of spin systems **5** and **6** in **3**, could be determined from the 2-D proton homonuclear COSY and 1-D homonuclear decoupling experiments. Coupling of the homonuclear and heteronuclear nmr correlation results for

Table 1. Carbon NMR Data for **1-4**.

C#	1		2		3		4	
1	40.9	bs	38.3	bd	39.0	d	39.3	d
2	67.2	d	67.1	d	68.3	d	66.6	d
3	145.9	s	145.6	s	145.9	s	145.8	s
4	121.7	s	122.0	s	121.3	s	122.0	s
5	109.7	d	109.8	d	109.8	d	109.0	d
6	149.3	s	149.4	s	149.4	s	149.0	s
7	56.3	d	56.3	d	56.2	d	57.0	d
8	53.3	s	56.2	s	56.1	s	55.7	s
9	39.2	t	39.2	bt	39.1	t	40.0	t
10	76.4	d	76.5	d	76.6	d	77.9	d
11	64.3	d	64.3	d	64.9	d	151.7[a]	s
12	60.8	s	60.9	s	61.4	s	134.1	d
13	69.9	d	69.9	d	68.5	d	69.0	d
14	28.6	bt	28.5	t	26.5	t	32.7	t
15	140.0	bs	149.6	s	57.1	s	150.0[a]	s
16	129.0	t	136.0	bt	50.7	t	137.0	bt
17	166.3	s	193.2	d	23.5	q	193.9	d
18	9.7	q	9.8	q	9.8	q	9.8	q
19	19.6	q	19.9	q	19.9	q	19.6	q
20	167.8	s	167.9	s	167.9	s	169.9	s
21	169.9[a]	s	169.6	s	170.1	s	169.7[b]	s
22	20.8[b]	q	20.4	q	21.2	q	20.5[c]	q
23	169.8[a]	s	169.8	s	169.9	s	170.4[b]	s
24	20.4[b]	q	20.7	q	21.0	q	20.8[c]	q
25	51.8	q						

[a,b,c] assignments interchangeable





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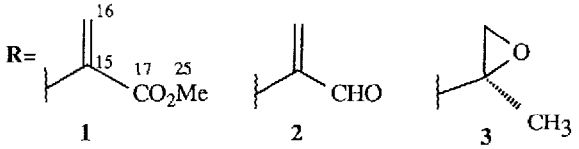

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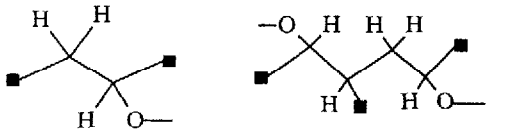

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Table 2. Proton NMR chemical shifts and multiplicities (J in Hz) for **1-4**.

H#	1			2			3			4		
1	4.53	bt	(10)	4.60	t	(10)	3.98	t	(11)	4.69	t	(10)
2	6.12	bd	(10)	6.02	d	(9)	5.74	d	(11)	6.03	d	(5)
5	6.00	s		6.00	s		5.97	s		5.88	s	
7	4.11	s		4.12	s		4.09	s		4.12	s	
9a	2.50	dd	(15, 3)	2.50	dd	(15, 3)	2.50	dd	(15, 3)	2.48	dd	(15, 4)
9b	2.05	dd	(15, 4)	2.08	m		2.04	dd	(15, 4)	2.14	dd	(15, 3)
10	4.76	t	(4)	4.76	t	(3)	4.75	dd	(4, 3)	5.19	m	
11	4.13	s		4.14	s		4.15	s		7.22	bs	
13	4.85	dd	(7, 1)	4.88	d	(3)	5.07	d	(5)	5.70	d	(6)
14a	2.84	ddd	(16, 7, 5)	2.80	m		1.89	ddd	(15, 11, 5)	2.69	ddd	(15, 10, 6)
14b	1.35	bd	(16)	1.39	d	(16)	0.98	d	(15)	1.04	d	(15)
16a	6.32	s		6.49	bs		2.75	d	(4)	6.63	bs	
16b	5.93	bs		6.16	bs		2.52	d	(4)	6.19	s	
17abc				9.55	s		1.59	s	(3H)	9.58	d	(1)
18abc	2.02	s	(3H)	2.01	s	(3H)	1.99	s	(3H)	2.01	s	(3H)
19abc	1.11	s	(3H)	1.10	s	(3H)	1.05	s	(3H)	0.97	s	(3H)
22abc	1.98[a]	s	(3H)	1.94	s	(3H)	2.18	s	(3H)	1.91	s	(3H)
24abc	1.95[a]	s	(3H)	1.87	s	(3H)	2.08	s	(3H)	1.91	s	(3H)
25abc	3.78	s	(3H)									

[a] assignments interchangeable

3, along with comparison of the nmr data to that of lophotoxin led us to suggest structure **3** for bipinnatin c. As a crystal suitable for x-ray diffraction analysis could be obtained from slow evaporation of a solution of **3** in methylene chloride/water/methanol, the structure was confirmed and the relative stereochemistry assigned by x-ray crystallography.

A crystal of bipinnatin b with measurements of 0.38 x 0.25 x 0.25 mm was used for the x-ray crystallographic study. Diffraction measurements were made on a Rigaku AFC5S fully automated diffractometer using graphite monochromated CuK_α radiation (λ=1.54178 Å). Preliminary indications of the unit cell based on 25 randomly selected reflections revealed orthorhombic symmetry. The data was processed using the high angle cell which gave the following lattice parameters: a=9.0285(8) Å, b=35.489(2) Å and c=7.2798(6) Å with $\alpha=\beta=\gamma=90.0°$. The space group was assigned as $P2_12_12_1$ (#19), Z=4 with one molecule of composition $C_{24}H_{28}O_{10}$ forming the asymmetric unit. The volume was 2332.5 (3) $Å^3$ and the calculated density was 1.36 g/cm^3. There were 2065 reflections collected with $2\Theta \leq 120°$, of those reflections, 1468 (71%) with $I \geq 3(\sigma I)$ were adjudged observed.

The structure of **3**, shown in Figure 1, was solved using SHELXS86[7]. The entire structure was located on the initial electron density map. Anisotropic refinement of the non-hydrogen atoms and inclusion of the hydrogen scattering factors have resulted in the convergence of the crystallographic reliability factors to R=0.045 and R_W=0.047. All intramolecular bond distances and bond angles are within normal range.

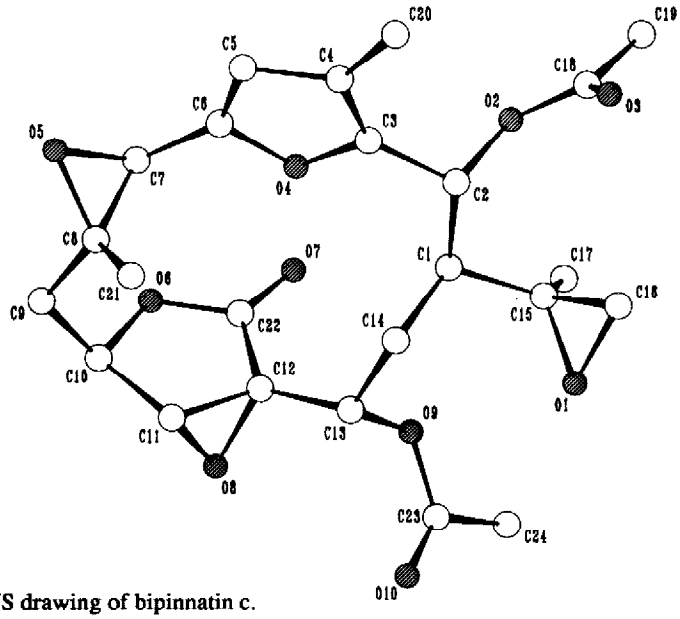

Figure 1. URANUS drawing of bipinnatin c.

Inspection of tables 1 and 2 suggests that bipinnatins a-d are very similar structurally. The major difference is the replacement of the methyl substituted epoxide (atoms 15, 16 and 17) observed in c with an α,β-unsaturated carbonyl moiety in both bipinnatins a and b. Bipinnatin a contains an α,β-unsaturated methyl ester with carbon resonances observed at 166.3 s, 140.0 s and 129.0 bt ppm and proton resonances observed at 6.32 bs, 5.93bs, and 3.78 (3Hs) ppm. The low field chemical shifts of the olefinic resonances and the long range carbon-hydrogen correlations between the olefinic protons and the carbonyl group of the ester support this

assignment. Bipinnatin b contains an α,β-unsaturated aldehyde with carbon nmr resonances observed at 193.2 d, 149.6 s and 136.0 bt ppm and proton resonancs observed at 9.55 s, 6.49 bs and 6.16 bs ppm, respectively. A multiple bond correlation between the aldehyde proton and the olefinic carbon resonance observed at 149.6 ppm supports this assignment. One bond and long range carbon hydrogen correlations as well as comparison of the carbon and proton chemical shifts and multiplicities with those of lophotoxin and **3**, suggested that structures **1** and **2** be assigned for bipinnatins a and b respectively. Bipinnatin d is most closely related to **2**. The major difference is the replacement of the α,β-epoxy-γ-lactone with an α,β-unsaturated-γ-lactone as evidenced by the carbon nmr resonances observed at 169.9 s, 151.7 s and 134.1 d ppm, the proton nmr resonance observed at 7.2 bs ppm and the loss of the ir absorbance observed at 1780 cm^{-1} in the ir spectra of **1-3**, suggesting that bipinnatin d has structure **4**. Bipinnatin c has the same relative sterochemistry as that reported for lophotoxin. Assignment of the relative stereochemistries of **1**,**2** and **4** is based upon comparison of the respective nmr chemical shifts and proton-proton coupling constants with those of **3**, lophotoxin and pukalide[8].

Bipinnatins a, b and d are active *in vitro* against the P388 murine tumor cell line with IC_{50}'s of 0.9, 3.2 and 1.5 μg/ml respectively. Bipinnatin c which lacks the α,β-unsaturated carbonyl functionality at C15-C17, is much less active with an IC_{50} of 46.6 μg/ml suggesting that this functionality enhances the activity of bipinnatins a, b and d. As both bipinnatin b and d show similar activities, the epoxide found at C11,C12 may not be essential for activity.

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References/Notes

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2. Bipinnatin a, **1**, $[\alpha]_d^{20}$= -76.6° ($CHCl_3$ c. 3.5); IR υ cm^{-1} 2950, 1780, 1725(b), 1420, 1360, 1200(b), 1090, 1030, 960, 920; Uv ($CHCl_3$) λ_{max}=286 nm (ϵ=10,500); EIMS: M^+ $C_{25}H_{28}O_{11}$ m/z_{obsvd}=504.1639 m/z_{calc}=504.1632, 462, 444, 193, 166, 137, 109, 93, 60. Bipinnatin b, **2**, $[\alpha]_D^{20}$=-68.9° (CH_2Cl_2 c. 1.2); IR: υ cm^{-1} ($CHCL_3$) 3000, 1785, 1735(b), 1420, 1370, 1210, 1085, 1020, 930(b), 820; Uv: ($CHCL_3$) λ_{max}=242 nm (ϵ= 948); EIMS: M^+ $C_{24}H_{26}O_{10}$ m/z_{obsvd}=474.1535, m/z_{calc}=474.1526, 432, 414, 372, 354, 137, 123, 109, 93, 60. Bipinnatin c, **3**, $[\alpha]_D^{20}$=-45.6° ($CHCL_3$ c. 0.54); IR: υ cm^{-1} 3000, 1780, 1730(b), 1420, 1370, 1210(b), 1080, 1030, 965, 925; Uv: ($CHCL_3$) λ_{max}=245 (ϵ =302); EIMS: $C_{24}H_{28}O_{10}$ m/z_{obsvd}=476.1688, m/z_{calc}=476.1683, 416, 326, 175, 137, 123, 109, 93, 60. Bipinnatin d, **4**, $[\alpha]_D^{20}$=+34.2° ($CHCl_3$ c. 0.17); IR: υ cm^{-1} 3000, 1750-1740, 1730, 1420, 1370, 1240, 1090, 1020, 960, Uv: ($CHCl_3$) λ_{max}=241 nm (ϵ=641), 230sh; EIMS: $C_{24}H_{26}O_9$ m/z_{obsvd}=458.1588; m/z_{calc}=458.1577, 398, 356, 338, 222, 180, 137, 84, 60, 49.
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