Lasonolides C−G, Five New Lasonolide Compounds from the Sponge *Forcepia* sp.†

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Five new marine-derived macrolide compounds, lasonolides C (**3**), D (**4**), E (**5**), F (**6**), and G (**7**), have been isolated from the sponge *Forcepia* sp. along with the parent compound in the series, lasonolide A (**1**). Their structures were elucidated on the basis of spectral data. Compounds **3**−**5** inhibit the in vitro proliferation of A-549 human lung adenocarcinoma cells with IC_{50}'s of 0.13, 4.5, and 0.31 μM, respectively. Compounds **3**−**6** inhibit the in vitro proliferation of PANC-1 human pancreatic carcinoma cells with IC_{50}'s of 0.38, 4.89, 0.57, and 15.6 μM, respectively. Compound **3** inhibits the in vitro proliferation of the NCI-ADR-RES cell line with an IC_{50} of 1.12 μM.

The marine sponge *Forcepia* sp. has been the source of the interesting macrolides lasonolides A (**1**)[1] and B (**2**).[2] These polyketide-derived macrolides have been reported to be potent inhibitors of cell proliferation in the A549 human lung adenocarcinoma cell line[1,2] and inhibit cell adhesion in the EL-4.IL2 cell line, a response that correlates with signal transduction activity.[3] Recently, the structure of lasonolide A has been revised on the basis of total synthesis.[4−8] As part of an effort to obtain sufficient lasonolide A for in vivo antitumor assay, multiple specimens of *Forcepia* were collected at a site in the U.S. Gulf of Mexico. HPLC analysis of the samples using PDA detection allowed for the detection of lasonolide A as well as a series of analogues that have the characteristic lasonolide chromophore. HPLC-guided fractionation of the crude extract led to the purification of five new lasonolide analogues, which we designate as lasonolides C (**3**), D (**4**), E (**5**), F (**6**), and G (**7**). All of the natural occurring compounds isolated to date possess the same C-1 through C-29 polyketide (which we have designated as the lasonopyran skeleton (Figure 1), but differ in the nature of the side chain substituents. This note describes the isolation, structure elucidation, and cytotoxicity of these new lasonolide analogues.

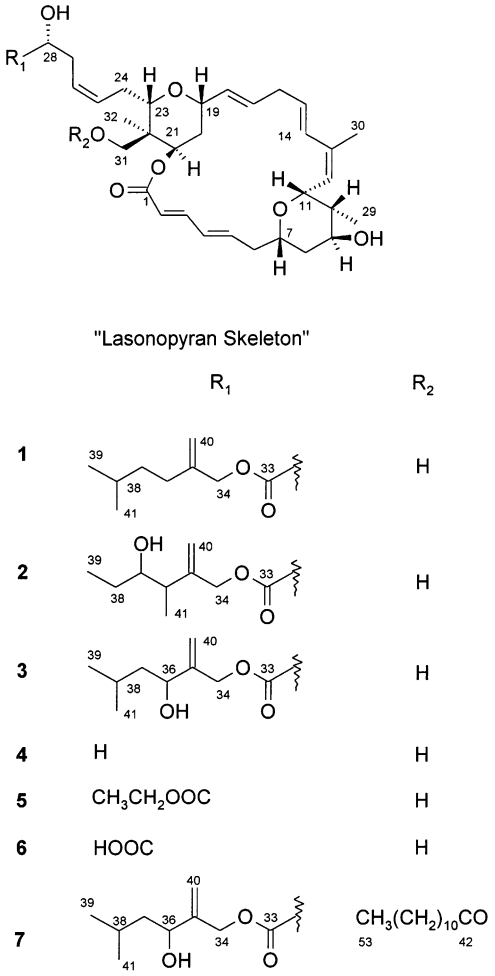

Figure 1. Structures of lasonolides A−G (**1**−**7**).

Results and Discussion

Samples of the sponge *Forcepia* sp. were collected using the Johnson-Sea Link human-occupied submersible at a depth of 70.7 m at a site located approximately 100 nautical miles southwest of Sanibel Island, FL, in the U.S. Gulf of Mexico. Samples for this study were stored at −20 °C immediately after collection. It was observed that lasonolide G (**7**) was unstable to slow freezing, and certain samples were flash frozen and stored at −20 °C to preserve the compound for chemical investigation.

Lasonolide C (**3**) was obtained as a white powder. The HRFABMS coupled with the ^{13}C NMR indicated a molecular formula of $C_{41}H_{60}O_{10}$ for **3**. All of the resonances attributable to the lasonopyran skeleton (C-1 to C-32) were present in the ^1H and ^{13}C NMR spectra of **3** (Table 1) and were virtually superimposable on those observed for lasonolide A (**1**). The differences observed in the NMR spectra corresponded to differences in the ester side chain (C-34−C-41). The most significant change was the loss of the resonance observed for the C-36 methylene group and the addition of resonances attributable to an oxygenated methine group [δ_H 4.24 m and δ_C 71.4 d], confirming the

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Table 1. NMR Spectral Data of Lasonolides A (**1**) and C (**3**)

	lasonolide A (**1**)		lasonolide C (**3**)			
no.	δ_H (*J* Hz)[a]	δ_C[b]	δ_H (*J* Hz)[a]	δ_C[b]	HMBC (H no.)	COSY
1		168.5 s		168.5 s	2, 3, 21	
2	5.71 d (15.4)	118.4 d	5.69 d (15.5)	118.2 d	3, 4	3
3	7.24 dd (15.4, 10.9)	148.3 d	7.24 dd (15.5, 11.0)	148.3 d	4, 5	2,4
4	6.24 dd (15.3, 10.9)	129.0 d	6.26 dd (15.0, 11.0)	129.0 d	2, 3, 5, 6	3,5
5	6.12 dt (15.3, 6.0)	145.1 d	6.15 dt (15.0, 6.0)	145.2 d	3, 4, 5, 6	4,6
6	2.36 m	38.6 t	2.35 m	38.5 t	4, 5, 7, 8a, 8b	5,7
7	4.06 m	72.5 d	4.05 m	72.5 d	5, 6, 8a, 9, 11	6,8a
8	1.69 m	33.8 t	1.66 m	33.7 t	6, 10	7, 8a, 9
	1.55 m		1.51 m			8a, 9
9	4.00 m	70.8 d	3.96 m	70.4 d	10, 29	8a, 8b, 10
10	1.66 m	38.4 d	1.64 m	38.2 d	11, 12, 29	9, 29
11	4.82 dd (9.2, 2.1)	69.0 d	4.82 dd (9.5, 1.7)	68.9 d	9, 12, 29	12
12	5.46 d (9.2)	124.6 d	5.46 d, (9.3)	124.6 d	11, 14, 30	11
13		139.0 s		138.8 s	11, 14, 15, 30	
14	6.59 d (15.7)	129.2 d	6.59 d (15.7)	129.1 d	12, 16ab, 30	15
15	5.83 dt (15.7, 7.4)	129.8 d	5.81 dt (15.7, 7.5)	129.6 d	14, 16ab	14, 16a
16	2.90 dd (12.3, 7.4)	33.7 t	2.89 dd (12.5, 7.5)	33.6 t	14, 15, 17, 18	15, 16b
	2.73 m		2.71 m			16a, 17
17	5.53 m	129.0 d	5.51 m	128.8 d	19,16ab	16b, 18
18	5.53 m	134.3 d	5.51 m	134.3 d	16ab, 19	17, 19
19	4.30 m	77.1 d	4.30 m	77.1 d	17, 18, 20a, 21, 23	18, 20a
20	1.89 dt (12.0, 2.5)	35.1 t	1.87 dt (13.0, <1)	34.9 t	21	19, 20a, 21
	1.42 dm (12.0)		1.40 m			20a, 21
21	4.97 brs	74.8 d	4.94 brs	74.7 d	20b, 31ab,	20a, 20b
22		41.3 s		41.2 s	20b, 21, 24a, 32, 31ab	
23	3.57 dd (10.8, 2.7)	78.0 d	3.56 dd (9.6, 2.2)	78.0 d	21, 24ab, 25, 31ab, 32	24a
24	2.20 m	28.1 t	2.22 m	28.1 t	23, 25, 26	23, 24b, 25
	2.06 m		2.07 m			24a, 25
25	5.69 dd (18.0, 7.5)	131.0 d	5.68 dd (15.0, 7.5)	130.8 d	23, 24ab, 26, 27	24ab, 26
26	5.50 m	125.2 d	5.49 m	125.0 d	24ab, 25,27,28	25, 27
27	2.52 m	32.6 t	2.50 m	32.5 t	25, 26, 28	26, 28
28	4.25 m	70.3 d	4.23 m	70.6 d	26, 27	27
29	1.06 d (7.1)	11.5 q	1.03 (d, (7.0)	11.4 q	10,11	10
30	1.82 s	21.1 q	1.80 s	21.0 q	12,14	
31	3.41 d (11.3)	65.7 t	3.39 d (11.5)	65.6 t	32	31b
	3.33 d (11.3)		3.32 d (11.5)			31a
32	1.11 s	15.2 q	1.08 (s)	15.1 q	23, 31ab	
33		174.0 s		173.9 s	27,28, 34ab	
34	4.62 s (2H)	67.7 t	4.76 d, (13.0)	64.7 t	36, 40ab	34b
			4.71 d (13.0)			
35		143.8 s		146.2 s	34ab, 36, 37ab, 40ab	
36	2.07 m	31.1 d	4.24 m	71.4 d	34ab, 37ab, 38, 40ab	37ab
37	1.34 m (2H)	36.7 t	1.49 m	44.6 t	36, 38, 39, 41	36a, 37b
			1.36 m			36a, 37a
38	1.56 m	27.8 d	1.74 m	24.6 d	36, 37ab, 39, 41	39
39	0.90 d (7.0)	22.5 q	0.93 d (6.8)[c]	22.0 q	37ab, 38, 41	38
40	5.03 s	112.5 t	5.24 bs	113.9 t	34ab, 36	40b
	4.97 s		5.15 bs			40a
41	0.90 d (7.0)	22.5 q	0.92 d (6.8)[c]	23.2 q	37ab, 38, 41	38

[a] ^{1}H spectrum recorded at 500 MHz in $CDCl_3$ referenced to residual C*H*Cl_3 (7.26 ppm). [b] ^{13}C spectrum recorded at 125 MHz in $CDCl_3$ referenced to *C*DCl_3 (77.0 ppm). [c] Assignments interchangeable.

presence of an additional alcohol functionality in **3**. Correlations observed in the HMBC spectrum between the olefinic protons observed at δ 5.24 and 5.15 (H40a and H40b) and the oxygenated methine carbon observed at 71.4 clearly allow for the assignment of the alcohol at C-36. The COSY spectrum indicated that the proton observed at δ 4.24 (H-36) was coupled to the methylene protons observed at δ 1.49 and 1.36 (H_2-37). The presence of an isopropyl functionality similar to that observed in lasonolide A was indicated by the presence of a methine proton observed at 1.74 (quintet J = 6.8 Hz, H-38), which showed strong coupling in the COSY spectrum to two methyl resonances observed at 0.93 (d, J = 6.8 Hz, H_3-39) and 0.92 (d, J = 6.8 Hz, H_3-41). No coupling was observed in the COSY spectrum of **3** between H_2-37 and H-38, but strong coupling observed in the HMBC spectrum between H_3-39 and H_3-41 and C-37 confirmed the placement of the isopropyl group. The close similarity of all coupling constants for protons in the lasonopyran portion of the molecule coupled with the similarity in rotation to that of **1** suggests that lasonolide C has the same relative stereochemistry as lasonolide A. The stereochemistry at C-36 has not been assigned.

Lasonolide D (**4**) was isolated as a colorless oil. Its molecular formula was deduced as $C_{32}H_{46}O_7$ from HR-FABMS and ^{13}C NMR data (Table 2). Inspection of the NMR data (^{1}H, ^{13}C) suggested that **4** possessed an intact lasonopyran skeleton. All of the resonances attributable to the ester side chain (C-33−C-41) were absent, suggesting that lasonolide D is a truncated form of lasonolide A. Interpretation of the various NMR spectra (COSY, HMQC, and HMBC) indicated the presence of a primary alcohol at C-28 (δ_H 3.67 dt, J = 10.5, 5.0 and 3.58 m; δ_C 61.8 t) instead of the oxygenated methine carbon observed for C-28 in **1** and **3**, thus suggesting that **4** is a descarboxy derivative of lasonolide A. The ^{1}H and ^{13}C spectra and rotation for **4** were similar to those observed for **1**, and

Table 2. NMR Spectral Data of Lasonolide D−F (**4**−**6**)

	lasonolide D (**4**)		lasonolide E (**5**)		lasonolide F (**6**)	
no.	δ_H (J Hz)[a]	δ_C[b]	δ_H (J Hz)[a]	δ_C[b]	δ_H (J Hz)[c]	δ_C[d]
1		168.5 s		168.5 s		169.0 s
2	5.71 d (15.5)	118.4 d	5.71 d (15.5)	118.3 d	5.75 d (15.2)	120.7 d
3	7.24 dd (15.5, 11.0)	148.3 d	7.24 dd (15.5, 10.5)	148.3 d	7.27 dd (15.2, 9.7)	148.0 d
4	6.26 dd (15.0, 11.0)	129.0 d	6.26 dd, 15.0, 11.0)	129.0 d	6.26 dd (15.0, 9.5)	130.5 d[e]
5	6.16 dt (15.0, 6.0)	145.1 d	6.15 dt (15.0, 6.0)	145.1 d	6.22 dt (15.0, 4.5)	145.4 d
6	2.36 m	38.5 t	2.36 m	38.5 t	2.22 m	40.5 t
7	4.06 m	72.5 d	4.06 m	72.5 d	4.01 t (11)	74.6 d
8	1.68 m	33.8 t	1.67 m	33.8 t	1.63 m	34.9 t
	1.53 m		1.53 m		1.47 m	
9	3.99 m	70.8 d	3.99 d (2.5)	70.8 d	3.84 m	71.4 d
10	1.60 m	38.4 d	1.64 m	38.3 d	1.58 m	39.1 d
11	4.81 dd (9.4, 1.8)	69.0 d	4.81 d (9.5)	68.9 d	4.81 dd (10, 2.5)	70.4 d
12	5.46 d (9.4)	124.6 d	5.46 d (9.5)	124.6 d	5.40 d (9.4)	125.9 d
13		138.9 s		138.9 s		139.7 s
14	6.58 d (16)	129.1 d	6.59 d (16)	129.1 d	6.62 d (15.9)	130.6 d[e]
15	5.82 dt (16, 7.5)	129.7 d	5.83 dt (16, 7.0)	129.7 d	5.84 dt (15.9, 8.0)	130.9 d[e]
16	2.90 m	33.7 t	2.90 dt (13.5, 7.0)	33.7 t	2.80 m	34.6 t
	2.76 m		2.75 m		2.68 m	
17	5.52 m	128.9 d	5.52 m	129.0 d	5.52 m	130.1 d[e]
18	5.52 m	134.2 d	5.50 m	134.2 d	5.50 m	136.0 d
19	4.32 dd (11, 7.0)	77.2 d	4.32 m	77.1 d	4.21 t (10.3)	78.6 d
20	1.86 t (13.0)	35.0 t	1.88 t (13.0)	35.0 t	1.81 dd (12.5, 5)	35.2 t
	1.42 m		1.42 m		1.29 m	
21	4.96 brs)	74.8 d	4.96 brs	74.7 d	5.01 brs	75.3 d
22		41.3 s		41.3 s		41.8 s
23	3.59 d (10.5)	77.8 d	3.56 dd (10, 2.5)	78.0 d	3.65 dd (10, 2.5)	80.9 d
24	2.33 m	28.0 t	2.20 m	28.1 t	2.30 m	29.4 t
	2.04 m		2.04 m		2.04 m	
25	5.62 m	130.8 d	5.68 m	130.8 d	5.58 m	130.3 d[e]
26	5.50 m	127.0 d	5.50 m	125.0 d	5.50 m	127.7 d
27	2.44 m	30.7 t	2.50 m, 2H	32.5 t	2.50 m	34.1 t
	2.25 m				2.30 m	
28	3.67 dt (10.5, 5)	61.8 t	4.20 dd (6.5, 4.5)	70.2 d	3.90 dd (6.7, 4.2)	73.2 d
	3.58 m					
29	1.06 d (7.0)	11.4 q	1.06 d (7.0)	11.4 q	0.99 d (7.4)	11.7 q
30	1.82 s	21.1 q	1.82 s	21.1 q	1.80 s	21.0 q
31	3.43 d (11.5)	65.7 t	3.41 d (11.5)	65.7 t	3.40 d (11.0)	66.1 t
	3.34 d (11.5)		3.33 d (11.5)		3.43 d (11.0)	
32	1.12 s	15.3 q	1.12 s	15.2 q	1.04 s	15.4 q
33				174.3 s		180.5 s
34			4.23 2H q (7.0)	61.5 t		
35			1.30 3H t (7.0)	14.2 q		

[a] ^{1}H spectrum recorded at 500 MHz in $CDCl_3$ referenced to residual C*H*Cl_3 (7.26 ppm). [b] ^{13}C spectrum recorded at 125 MHz in $CDCl_3$ referenced to *C*DCl_3 (77.0 ppm). [c] ^{1}H spectrum recorded at 500 MHz in CD_3OD referenced to residual C*H*$_3$OH (3.31 ppm). [d] ^{13}C spectrum recorded at 125 MHz in CD_3OD referenced to residual *C*D_3OD (49.0 ppm). [e] Overlapping resonances.

therefore it has been assigned to have the same relative stereochemistry as lasonolide A.

Lasonolide E (**5**) was isolated as a colorless oil. The HRFABMS coupled with the ^{13}C NMR spectrum suggested a molecular formula of $C_{35}H_{50}O_9$ for **5**. Comparison of the ^{1}H and ^{13}C spectra with those of compounds **1**−**3** suggested that **5** possessed the lasonopyran ring skeleton as observed for the previously identified compounds, but with a simplified ester side chain (Table 2). The observation of a methyl resonance [δ_H 1.30 t, J = 7.0 Hz; δ_C 14.2 q] that was mutually coupled to a oxygenated methylene group [δ_H 4.23 2H, q, J = 7.0 Hz; δ_C 61.5 t] suggested the presence of an ethyl ester in **5**. Long-range coupling observed between H_3-34 and the ester carbonyl observed at δ_C 174.3 confirmed the presence of the ethyl ester at C-33. It is possible that lasonolide E is an artifact from the isolation procedure.

Lasonolide F (**6**) was the most polar of the metabolites isolated from *Forcepia* sp. and was isolated as a colorless oil. The ^{13}C NMR and HRFABMS of **6** suggested a molecular formula of $C_{33}H_{46}O_9$. Comparison of the ^{1}H and ^{13}C NMR data including the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra suggested that **6** has an intact lasonopyran skeleton (Table 2). No resonances were observed for atoms C-34 and higher, suggesting that the ester functionality had been hydrolyzed to the free carboxylic acid. Consistent with this was the shift of the C-33 carbonyl resonance to δ_C 180.5.

Lasonolide G (**7**) was isolated as a light brown oil and was significantly more nonpolar than the other lasonolide analogues. The FAB mass spectrum ([M + H]$^+$, *m*/*z* 895) and the ^{13}C NMR spectrum led to the assignment of $C_{53}H_{82}O_{11}$ as the molecular formula. ^{1}H and ^{13}C NMR spectra showed the presence of characteristic resonances for the lasononopyran skeleton and for the C-34 to C-41 ester side chain as observed in lasonolide C (Table 3). The ^{13}C NMR spectrum indicated the presence of an additional ester carbonyl functionality (δ_C 173.8) as well as resonances indicative of a long chain aliphatic moiety in **7** [δ_C 34.0 t, 24.7 t, 32.6 t (multiple C), 31.9 t, 22.7 t, and 14.1 q]. The ester was placed on the primary alcohol at C-31 on the basis of a downfield shift of the protons attached to C-31 [δ 4.01 and 3.87 for H_2-31 in **7** vs δ 3.39 and 3.32 ppm in **3**]. This was further confirmed by the observations of long-range correlations between H31a and b and the new ester carbonyl resonance observed at δ_C 173.8 (C-42) in the HMBC spectrum of **7**. The number of CH_2 groups in the aliphatic chain attached to C-42 was deduced by the FABMS and is also supported by the ^{1}H and ^{13}C spectra.

Table 3. NMR Spectral Data of Lasonolide G (**7**)

no.	δ_H (*J* Hz)[a]	δ_C[b]	HMBC (H no.)
1		166.5 s	2, 3, 21
2	5.71 d (15)	120.0 d	
3	7.15 dd (15, 10.5)	145.9 d	4, 5
4	6.24 dd (15, 10.5)	129.3 d	2, 5
5	6.12 dt (15, 7.5)	143.4 d	3, 4, 6
6	2.30 m	39.3 t	4, 5
7	4.00 m	72.9 d	5, 6, 8b, 9
8	1.70 m	33.9 t	
	1.52 m		
9	3.98 m	70.9 d	10, 29
10	1.62 m	38.4 d	12, 29
11	4.78 m	69.0 d	9, 29
12	5.44 d (9.0)	124.7 d	11, 14, 30
13		138.5 s	11, 14, 15, 30
14	6.55 d (15.5)	129.5 d	16ab, 30
15	5.81 dt (15, 7.5)	129.3 d	17
16	2.88 dd (12.5, 7.0)	33.5 t	14, 15
	2.72 m		
17	5.45 m	128.4 d	19
18	5.45 m	135.1 d	16ab, 19, 20ab
19	4.24 m	77.5 d	17, 18, 20a, 21, 23
20	1.80 m	34.0 t	19
	1.39 m		
21	5.12 brs	72.7 d	32, 31b
22		39.3 s	32, 31ab
23	3.74 dd (10.5, 2.5)	78.3 d	24a, 31ab, 32
24	2.23 m	28.3 t	23, 26
	2.14 m		
25	5.72 m	130.7 d	23, 24ab, 26, 27
26	5.54 m	125.3 d	24ab, 27, 28
27	2.52 m	32.8 t	25, 28
28	4.26 m	70.4 d	27
29	1.05 d (7.0)	11.4 q	10, 11
30	1.81 s	21.0 q	12, 14
31	4.01 d (10.5)	66.7 t	32
	3.87 d (10.5)		
32	1.11 s	15.4 q	23, 31ab
33		174.0 s	27, 28, 34ab
34	4.79 d (13.2)	64.8 t	40ab
	4.73 d (13.2)		
35		146.3 s	34ab, 37a, 40ab
36	4.28 m	71.5 d	37ab, 40ab
37	1.53 m	44.7 t	36, 38, 39, 41
	1.39 m		
38	1.75 m	24.7 d	37ab, 39, 41
39	0.95 d (7.0)	22.1 q	37ab, 38
40	5.24 s	114.0 t	34ab
	5.17 s		
41	0.93 d (7.0)	23.2 q	
42		173.8 s	31ab, 43, 44
43	2.35 m	34.0 t	44
44	1.72 m	24.7 t	43, 45
45−50	1.32 m	32.6 t	
51	1.26 m	31.9 t	52, 53
52	1.30 m	22.7 t	51, 53
53	0.88 t (7.0)	14.1 q	51, 52

[a] ^{1}H spectrum recorded at 500 MHz in $CDCl_3$ referenced to residual $CHCl_3$ (7.26 ppm). [b] ^{13}C spectrum recorded at 125 MHz in $CDCl_3$ referenced to $CDCl_3$ (77.0 ppm).

Thus **7** is lasonolide C functionalized by a C-12 fatty acid ester at the C-31 position.

The lasonolide derivatives described herein represent an interesting comparison of the biological activity versus structure (Table 4) for this class of compound. All of the compounds have identical macrolide rings but vary substantially in the side chain substituents. (−)-Lasonolide A remains the most potent of the series and is 15-fold more active than the closest structural analogue (lasonolide C (**3**)) in the A549 cell line, 4.5-fold more active in the Panc-1 cell line, and 2-fold more active in the NCI-ADR/RES (formerly MCF-7/ADR) cell line. Interestingly lasonolides C and E, which vary significantly in the nature of the C-33 ester functionality, have similar cytotoxicity profiles in the A549 and Panc-1 cell lines but differ by at least 10-fold in the resistant cell line (NCI-ADR/RES). Hydrolysis of the ester to the free carboxy terminus (**6**) results in a significant loss of activity (>175-fold less active than lasonolide A). This could be due to limited transport of the more polar metabolite across the cell membrane. Functionalization by esterification with a long chain fatty acid at C-31 eliminates the cytotoxicity at concentrations measured during this study.

Table 4. Bioactivities of Lasonolides A (**1**) and C−G (**3**−**7**)

compound	A549[a] (IC_{50} μM)	PANC-1[b] (IC_{50} μM)	NCI/ADR-RES[c] (IC_{50} μM)
lasonolide A (**1**)	0.0086	0.089	0.49
lasonolide C (**3**)	0.13	0.38	1.12
lasonolide D (**4**)	4.50	4.89	>9
lasonolide E (**5**)	0.31	0.57	>8
lasonolide F (**6**)	>9	15.6	>9
lasonolide G (**7**)	>6	>6	>6

[a] A549 = human lung carcinoma. [b] PANC1 = human pancreatic carcinoma. [c] NCI/ADR-RES (formerly MCF-7/ADR).

During an expedition in 1999 to the Gulf of Mexico, it was observed that for the samples of *Forcepia* that contained lasonolide G, the concentration of lasonolide G would slowly decrease either during slow freezing of the specimen or holding of live specimens. The decrease in lasonolide G concentration corresponded with an increase in lasonolide C concentration, consistent with the action of an esterase to liberate the free C-31 alcohol. It is possible that lasonolide G serves as a storage compound in the organism, which can be easily hydrolyzed with native esterases to release the significantly more potent agent lasonolide C. The preparation of additional semisynthetic analogues is underway to provide additional insight into the active sites of the lasonolide nucleus.

Experimental Section

General Experimental Procedures. The IR spectra were collected on a Midac M-1200 with Galactic GRAMS/386 software. The UV spectra were collected on a Hitachi U-3010 spectrophotometer. The ^{1}H COSY and the ^{13}C, DEPT90, DEPT135, HMQC, and HMBC (optimized for 10 Hz) spectra were recorded on a Bruker AMX-500 operating at 500 MHz (^{1}H) and 125 MHz (^{13}C). Chemical shifts are referenced to solvent, e.g., $CDCl_3$; δ_H observed at 7.24 ppm and δ_C observed at 77.0 ppm or CD_3OD; δ_H observed at 3.31 ppm and δ_C observed at 49.0 ppm. The HRFABMS were measured using a Kratos MS50TC mass spectrometer.

Biological Material, Collection, and Identification. A sample of *Forcepia* sp. (phylum Porifera, class Demospongiae, order Poecilosclerida, family Myxillidae) was collected by manned submersible at a depth of 70.7 m in the Gulf of Mexico, 100 nautical miles west of Naples, FL (latitude 26°15.98′ N, longitude 83°42.58′ W). The sponge morphology is spherical with fat fingers protruding from the base. It is compressible, consolidates sediment at the base, and is red-orange in color both external and internal. The sponge corresponds most closely to the species *Forcepia triabilis*(?) as described by Van Soest.[8] A reference sample preserved in ethanol has been deposited in the Harbor Branch Oceanographic Museum (catalog number 003:01005, DBMR number 11-VIII-99-2-001) and is available upon request for taxonomic evaluation.

Extraction and Purification of Lasonolides. The frozen sponge (3.94 kg wet wt) was diced and exhaustively extracted with ethanol (Pharmco 100%). The combined ethanol extracts were concentrated to dryness, and the residue (86.5 g) was partitioned repeatedly between ethyl acetate and water. The organic partition was concentrated to dryness to yield 5.3 g of an orange oil. A 3.4 g sample of the residue from the organic

partition was chromatographed by vacuum flash chromatography on a RP-18 stationary phase (custom synthesized at HBOI) using a step gradient of $H_2O-CH_3CN-CH_3OH-CH_2Cl_2$ as eluent. The eluent series is as follows: fraction 1, H_2O-CH_3CN (8:2 v/v); fraction 2, H_2O-CH_3CN (1:1 v/v); fraction 3, H_2O-CH_3CN (2:8 v/v); fraction 4, CH_3CN; fraction 5, $MeOH-CH_2Cl_2$ (1:1 v/v). Fraction 1 (139.8 mg) was further purified by reversed-phase HPLC using a nonlinear gradient [Vydac Protein and Peptide C18 column, 1 cm i.d. × 25 cm, 10 μm particle size, solvent A: 5% CH_3CN in water v/v; solvent B: 100% CH_3CN; $t = 0$ min, A:B (85:15); $t = 10$ min, A:B (80:20); $t = 20$ min, A:B (70:30); $t = 25$ min, A:B (0:100); $t = 35$ min, A:B (0:100); flow = 3 mL/min; detected by UV absorption observed at 230 nm] to yield 8.6 mg of lasonolide F (**6**) (retention time = 17.05 min, 3.4×10^{-4} % of wet weight). Fraction 2 (314.5 mg) was further purified by reversed-phase HPLC [Vydac Protein and Peptide C18 column, 1 cm i.d. × 25 cm, 10 μm particle size, solvent A: 5% CH_3CN in water v/v; solvent B: 100% CH_3CN; $t = 0$ min, A:B (70:30); $t = 25$ min, A:B (25:75); $t = 30$ min, A:B (0:100); $t = 35$ A:B (0:100); flow = 3 mL/min; detected by UV absorption observed at 230 nm] to yield 48.9 mg of lasonolide C (**3**) (retention time = 16.75 min, 1.9×10^{-3} % of wet weight), 9.5 mg of lasonolide D (**4**) (retention time = 11.96 min, 3.7×10^{-4} % of wet weight), and 11.4 mg of lasonolide E (**5**) (retention time = 14.01 min, 4.5×10^{-4} % of wet weight). A 283 mg sample of fraction 3 was purified by reversed-phase HPLC [Vydac Protein and Peptide C18 column, 1 cm i.d. × 25 cm, solvent A: 5% CH_3CN in water v/v; solvent B: 100% CH_3CN; $t = 0$ min, A:B (50:50); $t = 25$ min A:B (20:80); $t = 35$ min A:B (0:100); $t = 40$ min, A:B (0:100); flow = 3 mL/min; detected by UV absorption observed at 230 nm] to yield 26.6 mg of lasonolide A (**1**) (retention time = 15.97 min, 1.0×10^{-3} % of wet weight).

Additional frozen sponge (2 kg wet wt) was diced and exhaustively extracted with ethanol. The combined ethanol extracts were concentrated to dryness (79.2 g) and partitioned between EtOAC and H_2O (1:1). The organic partition was further partitioned between 90% aqueous MeOH and heptane. After concentration by distillation under reduced pressure, 1.5 g of the 90% aqueous MeOH partition was obtained. A 1.2 g sample of this material was subjected to vacuum flash column chromatography on an RP-18 stationary phase using a step gradient of H_2O-CH_3CN as follows: fraction 1, H_2O-CH_3CN (8:2 v/v); fraction 2, H_2O-CH_3CN (2:8 v/v); fraction 3, CH_3CN (100%); and fraction 4, $MeOH-CH_2Cl_2$ (1:1 v/v). Fraction 3 (43.1 mg) was divided into two portions and chromatographed on a Waters C18 sep-pak column using a step gradient of fraction 1, H_2O-CH_3CN (4:6 v/v); fraction 2, H_2O-CH_3CN (2:8 v/v); fraction 3, CH_3CN (100%); and fraction 4, $MeOH-CH_2Cl_2$ (1:1 v/v). Fractions 1 and 2 were combined and purified by reversed-phase HPLC [Vydac Protein and Peptide C18 column, 1 cm i.d. × 25 cm, 10 μm particle size, solvent A: 5% CH_3CN in water v/v; solvent B: 100% acetonitrile; $t = 0-20$ min hold at A:B (10:90); $t = 25$ min A:B (0:100) then hold at 100% CH_3CN for 10 min; flow = 3 mL/min; detected by UV absorption observed at 230 nm] to yield 7.3 mg of lasonolide G (**7**) (retention time = 14.8 min, 4.6×10^{-4} % of wet weight).

Lasonolide C (3): white powder; $[\alpha]^{20}_D$ −9.8° (*c* 0.32 $CDCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 248 (4.2) 268 sh (4.1); IR (MeOH) ν_{max} 3420, 2921, 2870, 2855, 1735, 1689, 1632, 1596, 1468, 1386, 1258, 1016 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRFABMS M + H^+ *m/z* obsd 713.4273 (calcd for $C_{41}H_{61}O_{10}$ 713.4265).

Lasonolide D (4): colorless oil; $[\alpha]^{20}_D$ −5.3° (*c* 0.34 MeOH−$CDCl_3$, 1:1); UV (MeOH−$CHCl_3$, 1:1) λ_{max} (log ϵ) 248 (3.9) 268 sh (3.8); IR (MeOH) ν_{max} 3399, 2922, 2886, 1700, 1685, 1638, 1387, 1258, 1048, 1002, 971, 894 cm^{-1}; ^1H and ^{13}C NMR (Table 2); HRFABMS M^+ *m/z* 542.3258 obsd (542.3243 calcd for $C_{32}H_{46}O_7$).

Lasonolide E (5): colorless oil; $[\alpha]^{20}_D$ −18.0° (*c* 0.30 MeOH−$CDCl_3$, 1:1); UV (MeOH−$CHCl_3$, 1:1) λ_{max} (log ϵ) 248 (4.0) 268 sh (3.9); IR (MeOH) 3384, 2921, 2880, 2844, 1725, 1704, 1684, 1638, 1432, 1386, 1252, 1206, 1078, 1042, 1000, 965, 824 cm^{-1}; ^1H and ^{13}C NMR (Table 2); HRFABMS $[M + Na]^+$ *m/z* 637.3390 $[M + Na]^+$ (637.3352 calcd for $C_{35}H_{50}O_9Na$).

Lasonolide F (6): colorless oil; $[\alpha]^{20}_D$ −23.5° (*c* 0.23 MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.9) 268 sh (3.7); IR (MeOH) 3399, 2921, 1694, 1674, 1643 1591, 1437, 1381, 1273, 1181, 1078, 1047 cm^{-1}; ^1H and ^{13}C NMR (Table 2); HRFABMS $[M + Na]^+$ *m/z* 609.3038 obsd (609.3039 calcd for $C_{33}H_{46}O_9Na$).

Lasonolide G (7): tan oil; $[\alpha]^{20}_D$ −18.0° (*c* 0.50 MeOH); IR (MeOH) 3404, 2963, 2927, 2875, 2855, 1745, 1735, 1720, 1699, 1653, 1632, 1617, 1555, 1458, 1381, 1258, 1083 cm^{-1}; ^1H and ^{13}C NMR (Table 3); FABMS *m/z* 895 $[M + H]^+$.

Cytotoxicity Assays. The lasonolide compounds were analyzed as to their effects on proliferation of A549 human lung adenocarcinoma, PANC-1 human pancreatic cancer, and NCI-ADR-RES tumor cell lines. A549 human lung adenocarcinoma, PANC-1 pancreatic cancer cells, and the NCI/ADR-RES (formerly MCF-7/ADR) human breast cell lines were obtained from the American Type Culture Collection (Rockville, MD). Assays were run using protocols described previously. All samples were assayed a minimum of three times to derive the final IC_{50} value.[9]

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