Lasonolide A, A New Cytotoxic Macrolide from the Marine Sponge *Forcepia* sp.

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In our search for new antitumor agents from marine organisms, an extract from the shallow water Caribbean marine sponge, *Forcepia* sp.,[1] was discovered to inhibit the *in vitro* proliferation of A-549 human lung carcinoma cells as well as to inhibit cell adhesion in a newly developed whole cell assay[2] that detects signal transduction agents. The sponge metabolite responsible for the biological activity of the extract, which we have named lasonolide A,[3] represents a new class of marine-derived macrolides; reported herein are its isolation, structure elucidation, and biological profile.

A specimen of the sponge was collected in the British Virgin Islands.[4] The bioactive EtOH extract was solvent partitioned extensively, and a CH_2Cl_2 layer was chromatographed using reversed-phase vacuum flash column chromatography (ODS) and then Si gel HPLC.[5] Monitoring each fraction for cytotoxicity and inhibition of cell adhesion led to the isolation of lasonolide A (**1**, Figure 1) (2.3 mg from 100 g of frozen sponge).

Lasonolide A is a pale orange oil ($[\alpha]_D$ = +24.4° (*c* 0.045, $CDCl_3$)) whose molecular formula was deduced as $C_{41}H_{60}O_9$ from HRFABMS [$(M + H)^+$ *m/z* 697.4243, Δ 7.2 mmu] and NMR data (Table 1); the ^{13}C and DEPT[6] NMR spectra indicated 40 distinct resonances, which included two carbonyls, 14 olefinic carbons, nine oxygenated sp^3 carbons, and four methyl groups, resulting in a DEPT molecular formula of $C_{40}H_{54}$. Two degenerate methyl carbons are associated with the resonance observed at δ22.5; therefore, an additional CH_3 can be added to the DEPT formula. This molecular formula requires 12 degrees of unsaturation. Two degrees of unsaturation are accounted for by the presence of two carbonyl carbons. The 14 olefinic carbons account for an additional seven degrees of unsaturation. Therefore, based on the molecular and DEPT formulas, **1** must contain three rings as well as three exchangeable hydrogens. This is consistent with the IR spectrum of **1**, which exhibited a broad peak at 3425 cm^{-1}, indicating the presence of a hydroxyl functionality. The absorption peaks in the IR spectrum at 1736 and 1690 cm^{-1} indicate a conjugated ester and possibly an intramolecularly hydrogen-bonded ester, respectively.

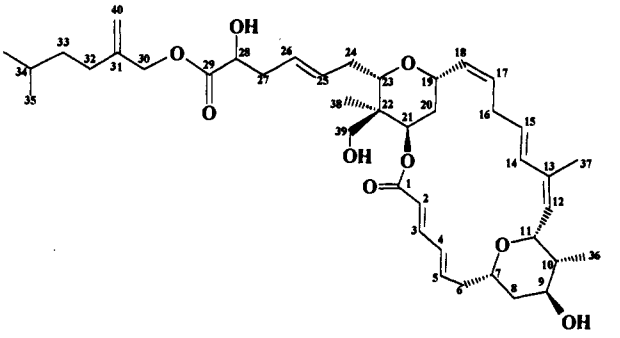

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Figure 1. Lasonolide A (**1**).

Table 1. NMR Data for Lasonolide A (**1**)

position	$^1H^a$	$^{13}C^{b,c}$	HMBC (H no.)
1		168.5 (s)	2, 3
2	5.71 (d, *J* = 15.4)	118.4 (d)	
3	7.24 (dd, *J* = 15.4, 10.9)	148.3 (d)	5
4	6.27 (dd, *J* = 15.3, 10.9)	129.0 (d)	2, 6
5	6.15 (dt, *J* = 15.3, 6.0)	145.1 (d)	3, 4, 6
6	2.36 (m)	38.6 (t)	
7	4.06 (m)	72.5 (d)	6, 8
8	1.69 (m)	33.8 (t)	
	1.55 (m)		
9	4.00 (m)	70.8 (d)	7, 36
10	1.66 (m)	38.4 (d)	36
11	4.82 (dd, *J* = 9.2, 2.1)	69.0 (d)	36
12	5.46 (d, *J* = 9.2)	124.6 (d)	11, 37
13		139.0 (s)	11, 15, 37
14	6.59 (d, *J* = 15.7)	129.2 (d)	12, 37
15	5.83 (dt, *J* = 15.7, 7.4)	129.8 (d)	16
16	2.90 (dd, *J* = 12.3, 7.4)	33.7 (t)	14, 15
	2.73 (m)		
17	5.53 (m)	129.0 (d)	
18	5.53 (m)	134.3 (d)	19
19	4.30 (m)	77.1 (d)	20, 21
20	1.89 (dt, *J* = 12.0, 2.5)	35.1 (t)	
	1.42 (dm, *J* = 12.0)		
21	4.97 (brs)	74.8 (d)	20, 38
22		41.3 (s)	38
23	3.57 (dd, *J* = 10.8, 2.7)	78.0 (d)	24, 38
24	2.20 (m)	28.1 (t)	
	2.06 (m)		
25	5.69 (dd, *J* = 18.0, 7.5)	131.0 (d)	23, 24, 27
26	5.50 (m)	125.2 (d)	27
27	2.52 (m)	32.6 (t)	
28	4.25 (m)	70.3 (d)	27
29		174.0 (s)	30
30	4.62 (s)	67.7 (t)	32, 40
31		143.8 (s)	30, 32
32	2.07 (m)	31.1 (t)	30, 33, 40
33	1.34 (m)	36.7 (t)	32, 35
34	1.56 (m)	27.8 (d)	32, 33, 35
35	0.90 (d, *J* = 6.8)	22.5 (q)[d]	33
36	1.06 (d, *J* = 7.1)	11.5 (q)	11
37	1.82 (s)	21.1 (q)	12, 14
38	1.11 (s)	15.2 (q)	23, 39
39	3.41 (d, *J* = 11.3)	65.7 (t)	38
	3.33 (d, *J* = 11.3)		
40	5.03 (s)	112.5 (t)	30, 32
	4.97 (s)		

[a] ^1H spectrum recorded at 500 MHz in $CDCl_3$, referenced to residual $CHCl_3$ (7.26 ppm). *J* in hertz. [b] ^{13}C recorded at 90 MHz in $CDCl_3$, referenced to $CDCl_3$ (77.0 ppm). [c] ^{13}C multiplicities determined in DEPT experiment. [d] Signal represents two degenerate carbons.

Extensive analysis of the ^1H–^1H COSY, 2-D HOHAHA,[7] and HMQC[8] spectra of **1** allowed for the generation of four

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(1) Per Van Soest, R. W. M. *Stud. Fauna Curacao Caribb. Isl.* **1984**, *66*, 1. The sponge has been identified as *Forcepia* sp.; the species may be *trilabis*. A taxonomic voucher specimen is deposited in the Harbor Branch Oceanographic Museum.

(2) Longley, R. E.; Harmody, D. *J. Antibiot.* **1991**, *44*, 93.

(3) The name lasonolide comes from the Phillipine word lason, which means poison or toxin.

(4) The sample (HBOI sample no. 1-VIII-92-1-1) was collected by scuba at a depth of 63 ft. on August 8, 1992, adjacent to Guana Island, British Virgin Islands (lat 18° 29.13′ N, long. 64° 33.65′ W); in life, the sponge is spherical and red-orange.

(5) The crude EtOH extract was partitioned between equal volumes of EtOAc and H_2O. The EtOAc-soluble material was further partitioned between 10% aqueous MeOH/heptane. Sufficient H_2O was added to the aqueous portion to bring the percentage to 40%. This was then extracted with equal portions of CH_2Cl_2. The CH_2Cl_2 partition was further purified via vacuum flash column chromatography using reversed-phase C-18 stationary phase and a solvent gradient of 1:1 MeOH/H_2O to 100% MeOH. Fractions obtained from 35% aqueous MeOH and 30% aqueous MeOH were combined and further purified by semipreparative HPLC (SiO_2, 7:3 EtOAc/heptane) to obtain pure **1**.

(6) Bendall, M. R.; Pegg, D. T.; Doddrell, D. M.; Williams, D. H. *J. Org. Chem.* **1982**, *47*, 3021.

(7) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355. The 2-D HOHAHA experiments were recorded with mixing times of 9, 20, and 35 msec.

(8) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.

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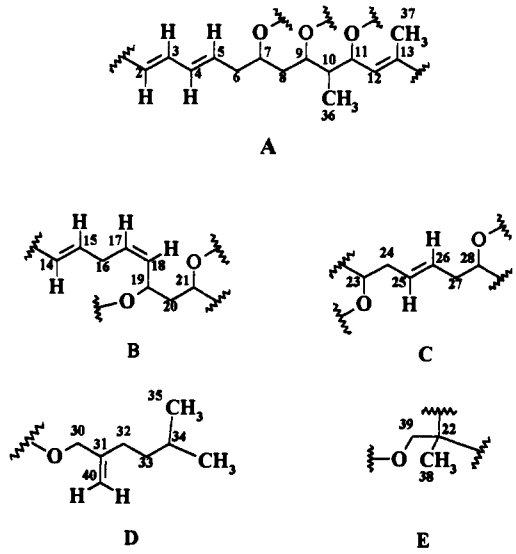

Figure 2. Lasonolide A substructures.

substructures A–D (Figure 2). Establishment of substructure E and connection of all five substructures and the remaining (carbonyl) carbons, C-1 and C-29, into the final gross structure of **1** were made primarily through interpretation of long-range ^1H–^{13}C correlations observed in an HMBC[9] spectrum (Table 1): the correlations observed between C-1 and H-2 and H-3 indicated that the carbonyl at C-1 is connected to fragment **A**. Fragment **A** was connected to fragment **B** on the basis of correlations observed between H(Me)-37 and C-12, C-13, and C-14, H-14 and C-37, and H-12 and C-14. Fragment **B** could then be extended to include fragment **E** by correlations observed between H(Me)-38 and C-21, C-22, and C-39 and to include fragment **C** on the basis of correlations observed between H(Me)-38 and C-23 and H-23 and C-38. The carbonyl at C-29 was connected, via the ester linkage, to C-30 on the basis of a correlation observed between H-30 and C-29. Because the remainder of the carbon skeleton of **1** has been accounted for completely, the carbonyl at C-29 must be connected to C-28; the chemical shift observed for H-28, i.e., δ4.25, corroborates this assignment.

Regio- and stereochemical assignments for the three hydroxyl groups and the ether linkages, the latter in the form of tetrahydropyran rings, were elucidated by a combination of ROESY[10] and deuterium exchange ^{13}C experiments:[11] cross peaks observed between H-7 and H-11 and H-19 and H-23 suggested not only that the respective carbons were linked via ether linkages resulting in two tetrahydropyran rings but also that these protons are configured in *cis*-1,3-diaxial relationships (Figure 3). The stereochemistry at C-7, C-9, C-10, and C-11 was further defined by ROESY cross peaks between H-9 and H(Me)-36 and H-8α, H(Me)-36 and H-12, and H-11 and H-10 and by the small ^1H–^1H coupling constant between H-10eq and H-11ax of 2.1 Hz. The stereochemistry of the second tetrahydropyran ring was further defined by ROESY cross peaks between H-20α and H(Me)-38, H(Me)-38 and H-21, and H(Me)-38 and H-24. Hydrogens associated with C-2 and C-3, C-4 and C-5, C-14 and C-15, and C-25 and C-26, exhibited coupling constants greater than 15 Hz suggesting trans stereochemistry for each olefin. Additional evidence for the trans orientation is obtained from ROESY cross peaks between H-2 and H-4, H-3 and H-5, and H-15 and H(Me)-37. The lack of through-space interactions between H-14 and H-15 and H-25 and H-26 gives further evidence

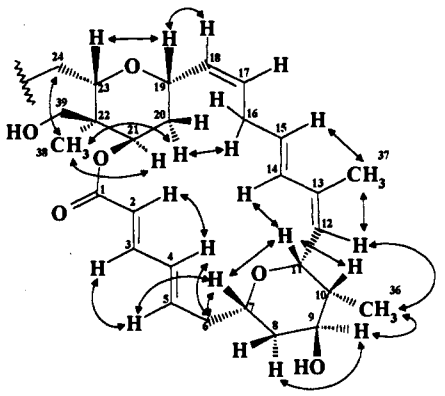

Figure 3. Lasonolide A ROESY correlations.

for their trans orientation. The C-12–C-13 double bond is assigned *Z* geometry as a result of an H-12 to H(Me)-37 ROESY cross peak. Although the proton resonances are partially overlapping, the C-17–C-18 double bond is assigned *Z* geometry on the basis of the absence of coupling constants greater than 10 Hz and on the basis of ROESY cross peak between H-20α (δ1.89) and one of the H-16 protons (δ2.90); the latter correlation could not be possible if the C-17–C-18 double bond in **1** is *E*.

The placement of the three hydroxyl groups on C-9, C-28, and C-39 and confirmation of the attachment of the C-1 ester to C-21 was made from analysis of results of a deuterium exchange experiment[11] that was performed by comparing ^{13}C chemical shifts for **1** recorded in CD_3OD to those recorded in CD_3OH. Carbons 9, 28, and 39 exhibited downfield shifts of greater than 0.1 ppm in CD_3OH, allowing for their assignment as hydroxyl-bearing carbons. Consistent with these assignments is the observance of a smaller chemical shift difference (less than 0.06 ppm) for those carbons (C-8, C-10, C-21, and C-27) α to the hydroxylated carbons. The remaining oxygen-bearing carbons (C-7, C-11, C-19, C-21, C-23, and C-30) exhibited no change in chemical shifts, nor did any of the other non-oxygenated carbons in compound **1**.

Similar to several other marine-derived macrolides, including tedanolide[12] and amphidinolide D,[13] lasonolide A is a potent cytotoxin, with IC_{50} values against the A-549 human lung carcinoma and P388 murine leukemia cell lines of 40 and 2 ng/mL, respectively. Further, **1** inhibits cell adhesion in the EL-4.IL-2 cell line with an IC_{50} of 19 ng/mL; however, toxicity against this cell line is greater than 25 μg/mL. Inhibition of cell adhesion correlates with signal transduction activity.[2] Additional biological evaluation of **1** is underway.

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Supplementary Material Available: NMR and IR data of **1** (29 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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