Antineoplastic agents. 257. Isolation and structure of Spongistatin 1.

ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · MARCH 1993

Impact Factor: 4.72 · DOI: 10.1021/jo00058a004

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Isolation and Structure of Spongistatin 1[1a]

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Received November 9, 1992

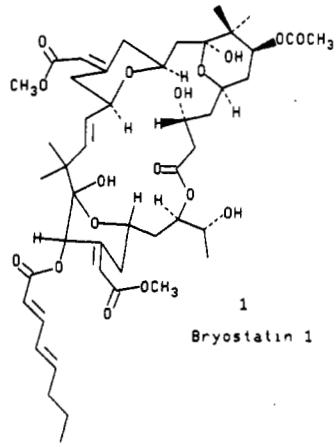
Summary: An Eastern Indian Ocean sponge in the genus *Spongia* contains a structurally unprecedented macrocyclic lactone, spongistatin 1 (**3**), with extremely potent activity against selected human tumor cell types in the U.S. National Cancer Institute's primary screen.

Early expectations and evidence that marine animals would prove to be productive sources of promising anticancer drugs bearing unprecedented structural architecture continues to be splendidly justified.[2,3] Bryostatin 1 (**1**) and halichondrin B (**2**)[4] represent two such examples of the macrocyclic lactone-type where perhydropyran rings represent the most prominent structural features. More recently, certain marine Porifera have been found to be very useful sources of pyran system macrocyclic lactones with cell growth inhibitory properties. Illustrative here are the misakinolide A[5] and swinholide A[6] series from marine sponges in the genus *Theonella*. In turn, these interesting compounds may be derived from symbiotic blue-green algae.[5b]

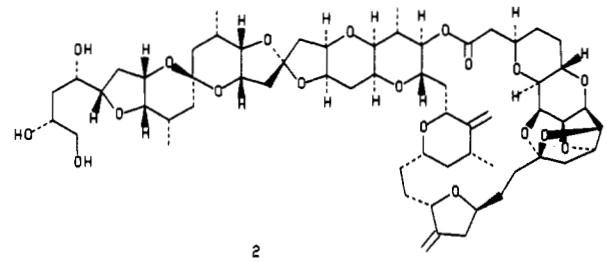
Marine Porifera in the genus *Spongia* (family Spongiidae, class Demospongiae) have proved to be good sources of tetracyclic diterpenes.[7] On the basis of previous investigations, the genus *Spongia* would not seem a particularly attractive reservoir of antineoplastic macrocyclic lactones, but natural products are replete with surprises. We are very pleased to report discovery in a *Spongia* sp. of a macrocyclic lactone designated spongistatin 1 that possesses a remarkable structure (**3**) exhibiting extraordinarily potent growth inhibitory activity against a distinctive subset of the U.S. National Cancer Institute's (NCI) panel of 60 human cancer cell lines.

A 1988 recollection (400 kg wet wt) of the dark brown (to black) *Spongia* sp. from the Eastern Indian Ocean (Republic of the Maldives, and originally located in our 1986 expedition) was extracted with methanol followed by dichloromethane–methanol. A dichloromethane fraction derived from the combined extract was subjected to a 9:1 → 3:2 methanol–water/hexane → dichloromethane solvent partition sequence. The final dichloromethane

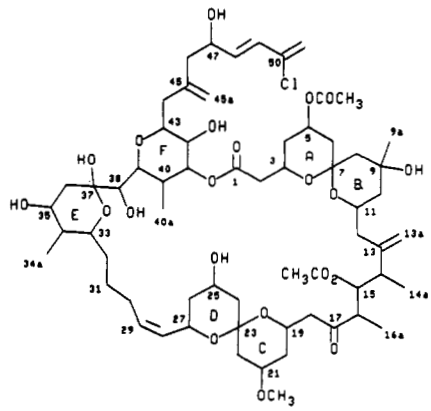
Chart I



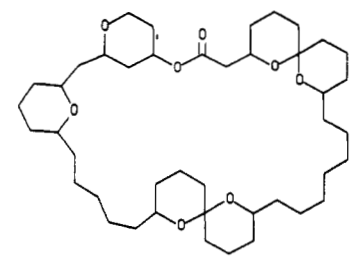
1
Bryostatin 1



2
Halichondrin B



3
Spongistatin 1



A
Spongipyran

0022-3263/93/1958-1302$04.00/0

Table I. NMR Assignments for Spongistatin 1 (3) Recorded in CD_3CN. Coupling Constants Are in Hz (in Parentheses). The Mixing Time for the HMBC Was Set at 130 ms

	^{13}C (100 MHz)	XH corr. (400 MHz)	HMBC (500 MHz, C to H)		^{13}C (100 MHz)	XH corr. (400 MHz)	HMBC (500 MHz, C to H)
1	173.07		H-2; H-41	30	28.07	2.00*; 2.19*	H-28; H-29; H-31; H-32
2	40.86	2.44 dd (10, 18)	H-4	31	27.04	1.23*; 1.60*	H-29; H-33; H-30; H-32
		2.53 dd (2, 18)		32	32.82	1.30 m; 1.42 m	H-33
3	63.59	4.25 brt (10)	H-2; H-8	33	67.15	4.13 dt (3.4, 3.4, 8)	H-34a
4	34.65	1.55*; 1.68*	H-2; H-6	34	39.32	1.57 m	H-34a; H-36
5	67.06	4.92 brs		34a	11.55	0.81 d (7)	H-33; H-34
6	38.17	1.67 dd (5, 14); 1.78 brd (14)	H-5; H-8	35	71.47	3.65 brs	H-34a; H-33; H-36
7	99.26		H-6; H-8; H-9a	36	33.79	1.61*; 1.89*	OH(C37); H-34
8	46.76	1.47 d (14); 1.60*	H-9a; H-6	37	99.41		H-33; H-36; OH(C37), H-38
9	69.64		H-9a; OH(C9); H-8	38	73.11	3.34 brs	H-36
9a	30.21	1.06 s	H-8; H-10	39	81.30	3.72 brd (10)	H-40a; H-41
10	44.96	1.28*; 1.55*	H-9a; H-12; H-8	40	37.26	1.91*	H-40a; H-39; H-41
11	65.00	4.25 brt (10)	H-12; H-13a; H-15; H-6	40a	12.69	0.74 d (7)	H-40; H-41
12	44.24	1.99*; 2.27 brd (14)	H-10; H-13a	41	80.60	4.75 dd (9, 11)	H-40a; H-39; H-40; H-42; H-43
13	148.03		H-12; H-13a; H-14a; H-15	42	73.11	3.12 t (9)	H-40; H-41; H-43; H-40a
13a	114.86	4.83 brs; 4.83 brs	H-12; H-14	43	78.72	3.39 brt (9)	H-39; H-41; H-42; H-44
14	36.60	2.78*	H-13a; H-14a; H-15; H-16; H-12	44	40.24	2.08*; 2.76 brd (13)	H-42; H-46; H-45a
				45	144.00		H-45a; H-43; H-44; H-46 H-47
14a	12.09	1.04 d (6.9)	H-15				
15	75.34	5.12 dd (1.7, 11)	H-13a; H-14a; H-16; H-16a	45a	116.61	4.86 brs; 4.89 brs	H-44; H-46
16	47.62	3.04 dq (7, 11)	H-15; H-16a	46	43.93	2.33 brdd (7, 14); 2.19*	H-44; H-45a
16a	13.73	1.15 d (7)	H-15; H-16	47	70.13	4.36 ddd (6, 7, 11)	H-46; H-48
17	213.52		H-16; H-16a; H-18; H-15	48	139.21	6.11 dd (6, 15)	H-46; H-47
18	51.94	2.62 brd (18); 2.86 dd (11, 18)	H-16; H-20	49	126.99	6.41 brd (15)	H-47; H-48; H-51
19	66.16	4.00 brt (11)	H-18	50	139.21		H-48; H-49; H-51
20	37.70	0.97 ddd (12, 12, 12); 1.98*	H-18; H-22	51	116.48	5.35 brs; 5.45 brs	H-48; H-49
21	73.98	3.46 tt (4, 4, 12, 12)	H-22; H-OMe; H-20	OMe	55.72	3.24 s	H-21
22	44.18	1.08 t (12); 1.99*	H-21; H-20	OAc	21.78	1.94 s	
23	99.91		H-18; H-22; H-24; H-27		171.61		H-OAc (δ 1.94); H-5
24	34.91	1.55*; 2.28*	H-22	OAc	21.00	1.84 s	
25	64.41	3.93 brm	H-26; H-27; H-24		170.21		H-OAc (δ 1.84); H-15
26	39.11	1.57*; 1.57*	H-28; H-24	OH(C25)			4.39 d (9.9)
27	61.22	5.00 ddd (4.3, 10, 10)	H-26; H-29	OH(C37)			4.73 d (2)
28	131.22	5.32 brt (10)	H-27; H-30	OH(C9)			4.32 brs
29	133.42	5.48 ddd (10, 10, 10)	H-27; H-30	OH			3.83 brm

* Coupling constants for these signals were not measured due to overlapping.

fraction was carefully separated (guided by P388 lymphocytic leukemia bioassay) employing an extensive series of LH-20 Sephadex gel permeation and partition (also on silica gel) chromatographic procedures, followed by final isolation using reversed-phase (Prepex 5–20 μm, C8 column) high-performance liquid chromatography with 5:5:7 acetonitrile–methanol–water as eluent to afford (13.8 mg, 3.4×10^{-7}% yield) colorless spongistatin 1 (3) as an amorphous powder, mp 161–162 °C: $[\alpha]^{22}_D$ +26.2° (c = 0.32, CH_3OH); UV (CH_3OH) λ_{max} 216 nm, ϵ 8490; IR (film) 3430, 2928, 1736, 1383, 1232, 1177, 1085, 993 cm^{-1}, high-resolution FAB MS, m/z 1245.5949 $[M + Na]^+$ corresponding to $C_{63}H_{95}ClO_{21}Na$ (calcd mass 1245.5952) with low-resolution FAB peaks at m/z 1245.5 $[M + Na]^+$ as parent peak and 1187.5 [M − 35] representing loss of chlorine.

Structural elucidation of spongistatin 1 (3) was especially challenging and required three separate (and in-depth), high-field 400- and 500-MHz 2D NMR analyses (APT, ^1H–^1H-COSY, ^1H–^{13}C-COSY, HMBC, and NOE) employing acetonitrile-d_3, pyridine-d_5, and methanol-d_4 as solvents. The assignments recorded in Table I are illustrative and require an extensive supporting discussion that will be reserved for a future report. Results of a series of selective acetylation experiments assisted in deducing some of these assignments. Due to the paucity of spongistatin 1 (3) presently available and its resistance to crystallization the stereochemistry of the chiral centers and their absolute configuration will require further investigation.

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Spongistatin 1 (3) was found to be extremely potent (GI_{50}'s typically 2.5–3.5 × 10^{-11} M) against a subset of highly chemoresistant tumor types (e.g., HL-60, SR leukemias; NCI-H226, NCI H23, NCI H460, NCI H522 non-small cell lung; DMS 114, and DMS 273 small cell lung; HCT-116, HT29, KM12, KM 20L2 and SW-620, colon; SF-539, U-251 brain; SK-MEL-5 melanoma; OVCAR-3 ovarian; and RXF-393 renal cancers) comprising the NCI panel of 60 human cancer cell lines.[8] Cell lines derived from human melanoma and lung, colon, and brain cancers were found to be especially sensitive to spongistatin 1 (3). The distinctive pattern of relative cellular sensitivity to spongistatin 1 was analyzed by computerized pattern-recognition algorithms and found to be closely correlated with the important general mechanistic class of microtubule-interactive antimitotics.[9] Spongistatin 1 represents the first member of a completely new class of cytostatic agents and may offer considerable promise for drug development research. Hence, spongipyran (A) is herein proposed for the new macrocyclic lactone ring system.

Extensive chemical and biological (e.g., in vivo human cancer xenograft experiments) studies of spongistatin 1 (3) and related *Spongia* constituents are in progress.

Acknowledgment. We are pleased to record the following very necessary financial support provided by Outstanding Investigator Grant CA 44344-01-03 and PHS grants CA-16049-07-12 awarded by the Division of Cancer Treatment, NCI, DHHS, the Fannie E. Rippel Foundation,, the Arizona Disease Control Research Commission, the Robert B. Dalton Endowment Fund, Virginia Piper, Eleanor W. Libby, L. Flugel, the Ladies Auxiliary, V.F.W., Dept. of Arizona, and the Fraternal Order of Eagles Art Ehrmann Cancer Fund. For other very useful assistance we are pleased to thank the Government of the Republic of the Maldives (Maizan H. Maniku, A. Naseer, and M. Shiham), Drs. Dennis L. Doubek, Fiona Hogan-Pierson, Ronald A. Nieman, and Michael D. Williams, Ms. Denise Nielsen Tackett, Mr. Larry P. Tackett, Mr. Lee Williams, the U.S. National Science Foundation (Grant CHE-8409644), and the NSF Regional Instrumentation Facility in Nebraska (Grant CHE-8620177).

Supplementary Material Available: High-field NMR spectra for spongistatin 1 (26 pages). This material is contained in 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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