THE STRUCTURE OF BRYOSTATIN 2 FROM THE MARINE BRYOZOAN *BUGULA NERITINA*[1]

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ABSTRACT.—The marine Bryozoan *Bugula neritina* L. has been found to contain a new series (17) of related macrolides remarkably active against the murine P-388 lymphocytic leukemia. Based primarily upon high resolution (400 MHz) ^{1}H-nmr studies, the second member of the series, bryostatin 2, has been shown to be the 20-membered ring lactone **1b**.

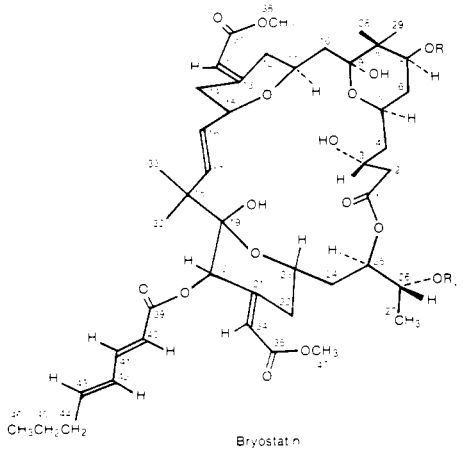
The sea-mat *Bugula neritina* (Linnaeus) enjoys a broad geographic range in the Atlantic, Pacific, and other ocean areas and is one of the more prominent Bryozoa species. In 1968 we found that certain Bryozoa members including *B. neritina* L. contain potentially important antineoplastic constituents (2). After a 14-year investigation we were able to describe the first isolation and structural elucidation of a Bryozoan anticancer component termed bryostatin 1 (**1a**, 3). The exciting inhibition (*e.g.*, 96% increase in life-span at a dose level of 70 μg/kg) shown by bryostatin 1 against the National Cancer Institute's murine P-388 lymphocytic leukemia (PS system), combined with its strikingly unique structure, led us to study related *B. neritina* biosynthetic products. Now we can report that an Eastern Pacific Ocean collection of the Bryozoan contains another remarkably potent (60% increase in life-span at 30 μg/kg) anticancer (PS system) constituent designated bryostatin 2 (**1b**). Furthermore, microgram quantities of 15 more exceptionally active (PS) bryostatins (designated 3-17) have been isolated from Pacific Ocean, Gulf of California, and Gulf of Mexico collections of *B. neritina* L.

While it is clear (spectral evidence) that bryostatins 2-17 are all derived from the bryopyran ring system (**2**)[2] only the 2 has so far been isolated in quantities sufficient for complete structure determination. Fortunately, a total of 314.5 mg ($\sim 6.2 \times 10^{-7}$% yield) of bryostatin 2 was obtained from 500 kg of wet *B. neritina* L., and it was possible to assign an unequivocal structure as follows: Bryostatin 2 was discovered employing the general methods already outlined for obtaining bryostatin 1 (3). In the final chromatographic (E. Merck prepack Size A silica gel columns, 10 psi) step, a solvent gradient from methylene chloride to methylene chloride-methanol (9:1) was used. Fractions (4 ml each) 98-109 crystallized from methanol-water. Recrystallization from methylene chloride-methanol afforded fine crystals melting at 201-3°: $C_{45}H_{66}O_{16}$; $[\alpha]^{25}D + 50°$ (c, 0.050, CH_3OH); λ max (CH_3OH) 230 (ε 36,250) and 261 (ε 35,600) nm; hr ei mass spectrum *m/e* 826 (M-2 H_2O), exact mass 826.4138 amu (calcd 826.4136 for $C_{45}H_{62}O_{14}$); ir (KBr), 3465, 2975-2950, 1715, 1700, 1640-1650, 1435, 1360, 1250, 1230, 1165, 1100, 1080, 1050 and 1000 cm^{-1}; and ^{1}H (see table 1), and ^{13}C-nmr (corresponding chemical shifts for bryostatin 1 are given in parentheses) 172.74 (172.29), (171.15), 167.12 (167.06), 166.89 (166.80), 165.66 (165.63), 156.95 (157.18), 152.01 (152.08), 146.49 (146.39), 145.58 (145.42), 139.18 (139.24), 129.65 (129.66), 128.45 (128.49), 119.68 (119.65), 118.64 (118.74), 114.32 (114.15), 101.90 (101.94), 99.11 (99.11), 79.16 (79.13), 74.12

[1]Part 89 of Antineoplastic Agents. For contribution 88 see reference (1).

[2]On the basis of very interesting aplasmomycin biosyntheses experiments using ^{13}C-labeled precursors by Floss (4), it appears likely that the bryopyran ring system (**2**) may arise from acetate units and the gem-dimethyl groups 28,29 and 32,33 from methionine.

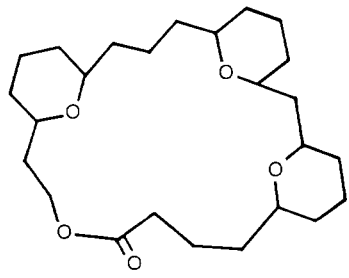
(74.19), 73.83 (73.73), (73.11), 71.65 (71.62), 70.12[3] (70.22), 68.50, (68.50), 66.06 (65.71), 64.76 (64.83), 51.11[3] (51.05)[3], 44.94 (44.94), 44.22 (44.29), 42.21 (42.31), 42.21 (42.02), 42.21 (41.11), 40.07 (40.00), 36.56 (36.56), 35.94 (36.04), 35.06[3] (35.09)[3], (33.44), 31.36 (31.42), 24.66 (24.66), 21.90 (21.90), 21.09 (21.15)[3], 19.76[3] (19.79), 15.60 (16.90), 13.68 (13.68).



Bryostatin

1a, R=CO-CH_3, R_1=H
1b R=R_1=H
1c R=R_1=$COCH_3$

As bryostatin 2 could not be obtained in a crystalline form suitable for X-ray crystal structure determination (3), unequivocal assignment of structure **1b** required analyses of the high resolution pmr spectra of bryostatins 1 and 2 supported by the results of ^{13}C-nmr ($CDCl_3$), mass, and other spectral investigations. The 400 MHz ^{1}H-nmr spectra of bryostatins 1 and 2 in $CDCl_3$ were tentatively assigned by extensive double resonance experiments (table 1). The proton spectrum of bryostatin 2 was nearly identical to that of bryostatin 1, except for the absence of the acetate methyl resonance at 2.05 ppm and the upfield shift of the C-7 proton from 5.15 to 3.95 ppm. These observations suggested that bryostatin 2 was the desacetyl analog of macrolide **1a**. The conclusion was strengthened by noting that the ^{13}C-nmr spectrum of **1b** did not differ significantly from that of **1a**, except for the loss of the acetyl carbonyl (171.15 ppm) and methyl (33.44) resonances, the upfield shift of an oxygen-bearing carbon (presumably C-7) from 73.11 to 70.12, the downfield shift of a quaternary carbon from 41.11 to



Bryopyran

2

[3]Signal may be two overlapping carbon signals.

TABLE 1. ^{1}H-nmr data for bryostatins 1 and 2.

Position	δ (ppm)		Multiplicity (J Hz)	
	1	2	1	2
2	2.45	2.45	m	m
3	4.09	4.13	m	m
4	1.55, 1.95	1.55, 1.95	m	m, m
5	4.19	4.13	m	m
6	1.4, 1.75	1.39, 1.58	m, m	m, m
7	5.15	3.95	m	m
10	2.1-2.2	2.1-2.2	m	m
11	~3.95	3.79	m	m
12	2.1-2.2	2.1-2.2	m	m
14	1.9, ~2.0	1.9, 2.0	m	m
15	4.08	4.02	m	m
16	5.30	5.29	dd(8.3, 15.9)	dd(8.3, 15.8)
17	5.76	5.75	d(15.9)	d(15.8)
20	5.16	5.16	s	s
22	~1.90	~1.90	m	m
23	~3.65	~3.65	m	m
24	1.95	1.95	m	m
25	5.19	5.12	m	m
26	3.73	3.78	m	m
27	1.23	1.21	d(6.3)	d(6.1)
28[a]	1.13	1.23	s	s
29[a]	0.98	1.01	s	s
30	5.66	5.65	s	s
32[a]	0.98	0.98	s	s
33[a]	0.92	0.88	s	s
34	5.98	5.98	s	s
37	2.05	——	s	——
38	3.68	3.68	s	s
40	5.80	5.78	d(15.3)	d(15.3)
41	7.26	7.24	m	m
42	6.16	6.14	m	m
43	6.16	6.14	m	m
44	~2.15	~2.15	m	m
45	1.42	1.42	m	m
46	0.90	0.89	t(7.3)	t(7.3)
47	3.64	3.64	s	s

[a]Assignments for these four groups may be interchanged.

42.21 (presumably C-8), and a small shift in the position of one of the gem-dimethyl group resonances (presumably C-28 or C-29).

Confirmation of the bryostatin 2 structure (**1b**) was obtained by a series of selective micro-scale acetylation-deacetylation experiments. The identical (tlc) acetate (**1c**, colorless needles from CH_2Cl_2-MeOH; mp, 249-250°) was obtained by partial acetylation (acetic anhydride-pyridine, 2 h, room temperature) of bryostatin 1 and 2. Careful deacetylation of acetate **1c** with hydrochloric acid (1%) in methanol (24 h, room temperature) or with potassium carbonate (1%) in methanol (24 h, room temperature) afforded a mixture of bryostatins 1 and 2. Because the structure of monoacetate **1a** was assigned by an X-ray crystal structure determination (3), the combination of evidence from high resolution nmr analyses and conversion to acetate **1c** established the structure (**1b**) of bryostatin 2.

Eventually, it may be possible to obtain sufficient amounts of the companion bryostatins 3-17 for definitive structure assignments. Meanwhile, bryostatin 2 is undergoing evaluation against a selection of the National Cancer Institute's experimental tumor systems. Because the bryostatins may prove to be important ionophores[4] for altering membrane permeability, other types of pharmacological (5,6) (*e.g.*, cardiovascular) and microbiological activities will also be explored. Discovery of the bryostatins and dolastatins (5) amply substantiates our early expectations (2) that the marine animals will prove to be an exceptionally productive and valuable source of new cancer chemotherapeutic drugs.

ACKNOWLEDGMENTS

We are very pleased to record financial assistance provided by Contract N01-CM-97262 with the Division of Cancer Treatment, NCI, National Institutes of Health, DHW, Grant Nos. CA16049-01 through 07 awarded by the National Cancer Institute, DHW; Mrs. Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.); the Fannie E. Rippel Foundation; Mrs. Eleanor W. Libby; the Donald Ware Waddell Foundation; Mrs. Pearl Spear; and Mr. Robert B. Dalton. Other very helpful assistance was provided by Drs. H. Cohen, D.L. Doubek, J.D. Douros, P.D. Ellis, J.L. Hartwell, L.W. Knapp, P. Lohavanijaya, M.I. Suffness, J.M. Schmidt, J. Witschel, Jr., Mr. M.A. Carlson, Miss B.L. Norfleet, Miss K.M. Welch, the Smithsonian Institution Oceanographic Sorting Center, and the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723).

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Received 13 September 1982

[4]Only the *Streptomyces griseus* cyclic ionophore aplasmomycin appears distantly related to bryostatins 1 and 2; see (7) and (8). Other such natural products seem only remotely related; see (9-16).