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ISOLATION AND STRUCTURE OF BRYOSTATINS 14 AND 15[1]

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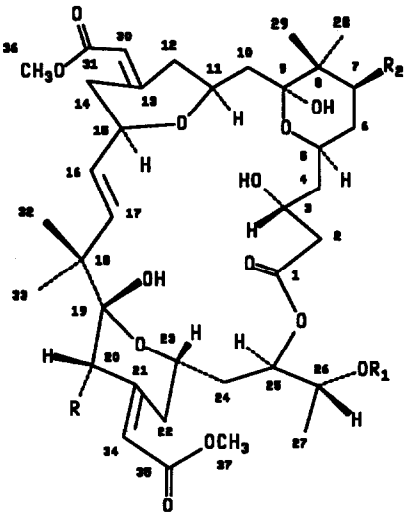
SUMMARY: Further investigation of constituents from the marine bryozoan *Bugula neritina* employing new 1,000 kg recollections from the Gulf of Mexico and Eastern Pacific Ocean (California) has led to isolation and structural determination of two previously undetected members of the bryostatin (1-13) series, bryostatins 14 (14) and 15 (15). Structural analyses were conducted primarily with high field (400 MHz) NMR and high resolution mass spectral techniques. Both new bryostatins significantly inhibited growth of the P388 lymphocytic leukemia.

Discovery[2] of bryostatins 1-13 (cf. 1-13) has made available a new class of important biochemical probes[3] with considerable clinical potential.[4] For example, with fresh samples of human myeloid leukemia, bryostatin 1 generally caused differentiation responses leading to macrophage-like morphology.[4a] Again, with peripheral blood cells from β-chronic lymphocytic leukemia patients, this substance triggered activation and differentiation[4b] and



STRUCTURE	R	R_1	R_2	
1	B	H	A	Bryostatin 1
2	B	H	OH	Bryostatin 2
4	D	H	C	Bryostatin 4
5	A	H	C	Bryostatin 5
6	A	H	D	Bryostatin 6
7	A	H	A	Bryostatin 7
8	D	H	D	Bryostatin 8
9	D	H	A	Bryostatin 9
10	H	H	C	Bryostatin 10
11	H	H	A	Bryostatin 11
12	B	H	D	Bryostatin 12
13	H	H	D	Bryostatin 13
14	OH	H	C	Bryostatin 14
14a	A	A	C	
15	E	H	A	Bryostatin 15

is undergoing clinical evaluation. In order to meet potential clinical supply requirements for the bryostatins, it became necessary to increase (to 1,000 kg, damp wt.) the size of *Bugula neritina* recollections from the Gulf of Mexico[2f] (Florida) and further explore such challenging quantities of biomass from the Eastern Pacific Ocean (California).[2i] We now report the isolation and structural elucidation of bryostatin 14 (14) from the Gulf of Mexico specimens in 1.02 x 10^{-5}% yield and bryostatin 15 (15) in 8.6 x 10^{-7}% yield from the Pacific Ocean collection of this remarkable bryozoan.

Bryostatins 4-8 (4-8) and 10 (10) were again[2f] isolated from the Gulf of Mexico recollection (1986) and this allowed application of contemporary NMR techniques (HMBC and NOE) to make some refinements in position assignments (Table 1). In turn, these two-dimensional NMR correlations aided the characterization of bryostatin 14 (14), separated from the previously known bryostatins by chromatography.

Bryostatin 14 (14, P388 ED_{50} 0.33 μg/mL) exhibited a FAB mass spectral base peak at m/z 831 $[M+Li]^+$ corresponding to molecular formula $C_{42}H_{64}O_{16}$. The EIMS of bryostatin 14 typically[2a,2h] did not show a molecular ion. Fragments at m/z 806, 788 and 770 suggested loss of three hydroxyl groups. The ^{1}H-NMR spectra of bryostatin 14 indicated the presence of the bryopyran ring.[2b] Both ^{1}H-^{1}H COSY and ^{1}H-^{13}C chemical shift correlation spectra allowed assignment of most ^{1}H and ^{13}C signals (Tables 2 and 3). Exceptions involved several overlapped ^{1}H-NMR signals and ^{13}C-NMR signals for carbons without proton bonds. A doublet at δ 75.13 clearly indicated a free hydroxyl group at the C-20 position. A pivalate was evident from the strong signals in the ^{1}H (δ 1.16, 9 protons) and ^{13}C (δ 27.14)-NMR spectra. The shift of the H-7 signal downfield to δ 5.10 suggested attachment of the pivalate group at C-7. Other ^{1}H and ^{13}C NMR signals were consistent with the assigned structure. However, a methyl singlet at δ 1.24 assigned to H-33 was at lower field than expected (Table 2). Unequivocal support for the bryostatin 14 structural assignment (14) was obtained by acetylation (acetic anhydride/pyridine) to afford diacetate 14a. As one result, the H-33 signal shifted upfield (from δ 1.24 in bryostatin 14 to δ 1.04 or 0.93 in acetate derivative 14a). Acetylation of bryostatin 5 (5) yielded a single product identical with diacetate 14a. Thus, the paramagnetic shift of the H-33 signal of bryostatin 14 was due to the C-20 hydroxyl group.

Additional evidence for the structure previously assigned bryostatin 5 and thence bryostatin 14 was obtained by HMBC (^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence)[5] NMR experiments that established attachment of the pivalate group at C-7, and unambiguous assignments for each of the carbonyl carbon atoms as well as C-8, C-9, C-13, C-18, C-19 and C-21 (Table 4). Finally, NOE difference spectroscopy was applied to assign the geminal dimethyl groups (C-28, 29 and C-32, 33). Irradiation of the H-28 signal at δ 0.91 enhanced the H-7 signal (at δ 5.10) and a broadened doublet signal at δ 1.64 (H-10α). Reciprocal irradiation of the H-7 signal enhanced the signal at δ 0.91. In contrast, irradiation of the signal at δ 0.98 enhanced only signals at δ 2.03 (doublet of doublets, H-10β) and 1.40 (H-6β). Therefore, the signal at δ 0.91 was assigned to the C-28 methyl and the signal at 0.98 to the C-29 methyl hydrogens. Irradiation of H-20 enhanced dramatically

Table 1. The ^{13}C-NMR chemical shift assignments for bryostatins 4, 5, 6, 8, and 10 recorded at 100.6 MHz, δ ppm in $CDCl_3$ solution.

	Bryostatin 4	Bryostatin 5	Bryostatin 6	Bryostatin 8	Bryostatin 10
C-1	172.29	172.37	172.48	172.24	172.66
2	42.14	42.19	42.17	42.30	42.14
3	68.46	68.53	68.48	68.47	68.15
4	39.91	39.96	39.85	39.87	39.78
5	65.48	65.56	65.62	65.72	65.63
6	33.19	33.24	33.38	33.38	33.20
7	72.64	72.66	72.71	72.54	72.61
8	41.20	41.26	41.04	41.02	41.27
9	101.73	101.79	101.85	101.82	101.79
10	41.86	41.92	41.91	41.96	42.05
11	71.45	71.51	71.54	71.51	71.35
12	44.13	44.17	44.16	44.14	44.19
13	157.23	157.18	157.06	156.71	157.07
14	36.48	36.46	36.44	36.37	36.57
15	78.91	78.98	79.04	79.08	78.95
16	129.66	129.73	129.68	129.56	130.45
17	138.97	138.96	138.96	139.13	137.90
18	44.75	44.80	44.80	44.82	44.75
19	98.84	98.85	98.83	98.89	100.94
20	74.24	74.45	74.42	74.24	39.78
21	151.83	151.73	151.68	151.81	157.03
22	31.20	31.23	31.20	31.22	36.11
23	64.70	64.74	64.72	64.71	64.62
24	35.81	35.85	35.85	35.89	35.73
25	73.59	73.67	73.76	73.69	73.84
26	70.03	70.09	70.13	70.18	70.21
27	19.83	19.70	19.66	19.78	19.66
28	21.00	21.06	21.08	21.06	21.06
29	16.92	16.98	16.92	16.88	17.06
30	113.99	114.10	114.14	114.31	114.19
31	166.73	166.79	166.81	166.72	166.82
32	19.78	19.86	19.71	19.78	20.39
33	24.57	24.63	24.60	24.57	24.45
34	119.59	119.73	119.72	119.63	115.73
35	166.98	167.00	166.99	166.99	167.00
36	51.08	51.16	51.13	51.07	51.07
37	51.01	51.07	51.07	51.01	50.84
R_2 1'	178.32	178.32	173.60	173.40	178.14
2'	39.08	39.07	36.56	36.54	39.05
3'	27.09	27.15	18.55	18.54	27.16
4'	27.09	27.15	13.66	13.65	27.16
5'	27.09	27.15			27.16
R 1"	172.00	169.37	169.36	172.00	
2"	36.41	21.49	21.48	36.54	
3"	18.17			18.22	
4"	13.58			13.61	

Table 2. The ^1H NMR data for compounds 14, 14a and 15 recorded at 400 MHz in $CDCl_3$ (some J values were measured with J-resolved 2D NMR).

	14	14a	15	15
H- 2	2.52 t(11)	2.39 dd(12.5,14)	2.45 brs	R
2	2.42 dd(2.2,11)	2.37 d(14)	2.45 brs	2" 5.93 d(15.4)
3	4.12 brd(11)	4.08[a]	4.16 m	3" 7.24 m
4	1.97 brdd(11,15)	1.95[a]	1.75 m	4" 6.39 dd(11,15.5)
4	1.54 brd(15)	1.53 dt(15,2)	1.60 m	5" 6.07 dd(7.5,15.5)
5	4.20 brt(11)	4.18 brt(11)	4.20 brt(10)	6" 4.38 q(7.5)
6α	1.67 brdd(5,12)	1.68 brdd(5,12)	1.77 m	7" 1.59 m
6β	1.40 dt(12,12)	1.38 dt(12,12)	1.48 m	1.72 m
7α	5.10 dd(5,12)	5.05 dd(5,12)	5.14 dd(5,12)	8" 0.94 t(7.5)
10α	1.64 brd(15)	1.62 brd(15)	1.65 m	
10β	2.03 dd(8,15)	2.03 dd(8,15)	2.07 m	
11	3.91 ddd(4,8,13)	3.78 brdd(8,13)	3.81 m	
12	2.15 brt(13)	2.15 brt(13)	2.21 brt(13)	
12	2.05 dd(8,14)	2.0[a]	2.07[a]	
14	3.65 brd(14)	3.58 brd(14)	3.68[a]	
14	1.90 brdd(12,14)	1.85 brdd(12,14)	1.96 brt(12)	
15	4.02 brdd(8.5,12)	4.05 brdd(8.5,12)	4.07 m	
16	5.30 dd(8.5,16)	5.24 dd(8.5,16)	5.29 dd(8.5,16)	
17	5.75 d(16)	5.71 d(16)	5.78 d(16)	
20	3.89 d(7.5)	5.04 s	5.21 s	
22	3.68[a]	3.62[a]	3.68[a]	
22	2.15 brdd(11,13)	1.98 brdd(11,13)	2.10 m	
23	3.98 brt(11)	3.93 brt(11)	4.01 m	
24	1.86[a]	1.82[a]	1.81 m	
24	1.80 brdd(11,14)	1.72 ddd(3,11,13)	1.81 m	
25	5.12 brm	5.25 brm	5.20 m	
26	3.74 dq(7,6.5)	4.95 dq(7,6.5)	3.81 m	
27	1.18 d(6.5)	1.16 d(6.5)	1.23 d(6.5)	
28	0.91 s	0.95[b]s	0.94[b]s	
29	0.98 s	0.88[b]s	0.99[b]s	
30	5.65 brs	5.62 brs	5.68 brs	
32	1.12 s	0.93[b]s	0.99[b]s	
33	1.24 s	1.04[b]s	1.14[b]s	
34	5.77 brs	5.91 d(1.8)	6.00 brs	
36	3.68 s	3.64 s	3.69 s	
37	3.66 s	3.61 s	3.66 s	
R_2 3'	1.16 s	1.13 s		
4'	1.16 s	1.13 s		
5'	1.16 s	1.13 s		
20-OH	4.22 d(7.5)	2.01 s(R_1)	2.04 s(R_2)	
		2.09 s(R)		

a, Couplings obscured due to overlapping.
b, Assignments for these signals may be interchanged.

Table 3. The ^{13}C-NMR assignments for bryostatin 14 (14), derived diacetate (14a) and bryostatin 15 (15) recorded at 100.6 MHz, δ ppm in $CDCl_3$ solution; The n (negative, 3 or 1 protons) and p (positive, 2 or no protons) are APT results.

	14	14a	15		15
C-1	172.57p	170.82	172.24	R	
2	42.25p	42.21	41.95	1"	165.10
3	68.53n	68.60	68.48	2"	121.75
4	40.00p	39.98	39.87	3"	144.58
5	65.58n	65.83	65.73	4"	130.82
6	33.24p	33.20	33.34	5"	141.39
7	72.62n	71.47	72.88	6"	86.86
8	41.25p	41.18	40.98	7"	25.44
9	101.76p	101.76	101.83	8"	9.57
10	41.92p	41.96	42.28		
11	71.48n	72.14	71.49		
12	44.11p	44.05	44.14		
13	156.70p	156.06	156.67		
14	36.46p	36.33	36.36		
15	79.01n	79.03	79.08		
16	129.56n	129.54	129.58		
17	139.15n	139.21	139.09		
18	44.93p	44.77	44.88		
19	99.38p	98.88	98.95		
20	75.13n	71.47	74.33		
21	156.94p	151.63	151.73		
22	30.56p	31.13	31.30		
23	64.40n	64.60	64.72		
24	35.78p	35.90	35.84		
25	73.74n	74.32	73.63		
26	70.04n	70.35	67.00		
27	19.54n	19.88	19.80		
28	21.02n	21.01	21.14		
29	16.93n	16.85	16.82		
30	114.23n	114.63	114.32		
31	166.72p	167.00	166.72		
32	19.88n	16.49	24.65		
33	24.66n	24.50	29.69		
34	116.69n	119.68	119.74		
35	167.09p	166.64	166.98		
36	51.04n	51.08	51.05		
37	51.04n	51.05	51.05		
R_2 1'	178.23p	177.93			
2'	39.03p	39.01			
3'-5'	27.14n	27.15			
R_1		170.30;21.47	170.99;21.06		
R		169.28;21.18			

Table 4. Bryostatin 14 (14) ^{1}H- and ^{13}C multiple bond correlations (HMBC) recorded at 500 MHz in $CDCl_3$ solution.[a]

Proton position		Proton position	
H- 2	C-1, C-3, C-4	22	C-21, C-23, C-24, C-34
4	C-2	23	C-22
4	C-2	24	C-23
5	C-3	26	C-24, C-25, C27
6	C-5, C-8	27	C-25, C-26
6	C-4, C-5, C-8	28	C-7, C-8, C-9, C-29
7	C-1', C-6, C-8, C-28, C-29	29	C-7, C-8, C-9, C-28
10	C-8, C-9, C-11, C-12	30	C-12, C-13, C-14, C-31
10	C-9, C-11	32	C-17, C-18, C-19, C-33
12	C-11, C-13, C-14, C-30	33	C-17, C-18, C-19, C-32
12	C-11, C-13, C-14, C-30	34	C-20, C-21, C-22, C-35
14	C-13	36	C-31
14	C-13, C-15, C-16, C-30	37	C-35
15	C-17	R_2	
16	C-14, C-18	3'-5'	C-1', C-2'
17	C-15, C-18, C-19, C-32, C-33	C-9 OH	C-9
20	C-21, C-34	C-19 OH	C-18, C-19, C-20
22	C-21, C-20, C-34	C-20 OH	C-19, C-20

a, Underlined positions correspond to weak signals; H-3' correlated also with C-4', 5'; H-4' with C-3', 5' and H-5' with C-3', 4'.

the H-34 signal at δ 5.77 and to some extent the methyl signal at δ 1.12, but not at δ 1.24. In keeping with this result, irradiation of the signal at δ 1.12 (C-32 hydrogen) enhanced the signals for H-20 (δ 3.89) and H-16 (δ 5.30) whereas irradiation of the H-33 methyl signal (at δ 1.24) increased the H-17 signal (δ 5.75). Thus, the methyl hydrogen signals at δ 1.12 and 1.24 were assigned respectively to C-32 and C-33.

For reisolation of bryostatins 1 (1) and 2 (2) from a more recent (1987) recollection (~1,000 kg, damp wt) of California *Bugula neritina* we initiated separation of the crude extract as previously described[21] and then devised a very useful high speed countercurrent distribution (HSCCD)[6] technique followed by further separation using HPLC and recrystallization to yield 8.6 mg (8.6 x 10^{-7}% yield) of bryostatin 15 (15, P388 ED_{50} 1.4 μg/mL).

The FAB mass spectrum of bryostatin 15 gave $[M+Li]^+$ at m/z 927 corresponding to molecular formula $C_{47}H_{68}O_{18}$ (16 mass units more than bryostatin 1 at mol. wt. 904). The ^1H NMR (400 MHz) spectrum revealed a macrocyclic lactone possessing an octadienoate side chain similar to bryostatin 1 (1). But chemical shifts of hydrogen signals in the olefinic region suggested a substitution change in the octadienoate side chain. The C-4" hydrogen signal of this ester appeared at δ 6.39 (dd, J 15.5, 11 Hz) and the C-5" at δ 6.07 (dd, J 15.5, 7.5 Hz) downfield compared to their counterparts in bryostatin 1 at δ 6.16 (dd, J 8.5, 2.4 and 4.8, 1.5 Hz respectively). At other positions in the C-20 ester the 2" hydrogen signal showed a doublet at δ 5.93 (J 15.4 Hz). The 3" hydrogen appeared slightly downfield at δ 7.28 (multiplet) compared to that of bryostatin 1 at δ 7.25 (multiplet). These observations were further supported and confirmed by 2D COSY and ^{13}C NMR experiments which led to a firm structure assignment for bryostatin 15 (15).

The biosynthetic processes orchestrated by *Bugula neritina* have produced a very useful series of bryostatins for detailed structure/activity studies. Whether by endogenous and/or exogenous biosyntheses, we now have a number of subtle structural modifications in hand that would be very difficult to realize by total[7] or semisyntheses. In turn, further biological evaluation of these substances should provide important insights for future anticancer drug design.

EXPERIMENTAL

GENERAL PROCEDURES. Solvents used for column chromatography were freshly distilled. Sephadex LH-20, particle size 25-100 μm, used in gel permeation and partition column chromatographic separations was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. The P.C. Inc. Ito-Multilayer Coil Separator-Extractor Model 1 was employed for high speed countercurrent distribution (HSCCD). An FMI lab pump Model RP SYX (Fluid Metering Inc., Oyster Bay, N.Y.) delivered the mobile phase and fractions were collected using Gilson FC-220 race track and FC-80 microfractionators. Thin layer chromatography silica gel plates were obtained from Analtech, Inc. The TLC plates were viewed under shortwave UV-light and then developed by 20% sulfuric acid and/or anisaldehyde-acetic acid spray reagent followed by heating at approximately 150°C. Uncorrected melting points were observed with a Kofler-type mp apparatus. Optical rotations were determined employing a Perkin-Elmer Model 241

polarimeter. IR spectra were recorded with a Nicolet MX-1 FT-IR Spectrometer and UV spectra were obtained by using a Hewlett-Packard 8450 UV-VIS spectrometer. In high pressure liquid chromatography separations Phenomenex Prepex (particle size 5-20 μ, ϕ 10.0 mm x 25 cm) C-8 was used in reversed phase mode and Phenomenex Prepex (particle size 5-20 μ, ϕ 10.0 mm x 25 cm) silica gel was used in normal phase mode using Altex (Model 110A) solvent metering pumps and Gilson HM UV detection at 254 nm. The ^{1}H NMR, ^{13}C NMR, 2D COSY, ^{1}H-^{13}C correlation, and NOE were recorded with a Bruker AM-400 instrument equipped with cryomagnet and ASPECT-3000 computer. The HMBC data were recorded using a Varian 500 NMR spectrometer. Mass spectra (70 eV and FAB) were obtained employing a Kratos MS-50 spectrometer.

Bryostatin 14 (14). Approximately 1,000 kg (damp wt.) of *Bugula neritina* was recollected in May, 1986 in the Gulf of Mexico near Florida, USA. The animal was preserved in 2-propanol and subjected to separation as previously discussed for a 50 kg, 1984 collection.[2f] The methylene chloride fraction from the solvent partition sequence was diluted with a mixture of ethyl acetate-2-propanol-water (12:1:6, 55 L). Separation of the organic phase (33.6 L) yielded 906.5 g of P388 lymphocytic leukemia cell line active fraction which was subjected to a series of steric exclusion and partition column chromatographic steps using Sephadex LH-20 and silica gel, similar to those previously described.[2f] Further purification was performed with high speed countercurrent distribution employing hexane-ethyl acetate-methanol-water (3:7:5:5), with the upper layer as mobile phase and lower layer as stationary phase (detailed below for bryostatin 15) followed by HPLC using hexane-isopropanol (9:1) in normal phase and methanol-water (4:1) in reversed phase. By these techniques the known bryostatin 4 (306 mg, 3.06 x10^{-5}% yield),[2d] bryostatin 5 (187 mg, 1.87 x 10^{-5}% yield),[2e] bryostatin 6 (32.5 mg, 3.25 x 10^{-6}% yield),[2f] bryostatin 7 (3.1 mg, 3.1 x 10^{-7}% yield),[2f] bryostatin 8 (23.5 mg, 2.35 x 10^{-6}% yield),[2f] and bryostatin 10 (39.0 mg, 3.9 x 10^{-6}% yield)[2h] were obtained accompanied by 102 mg (1.02 x 10^{-5}% yield) of the new bryostatin 14 (14) as an amorphous powder: mp 174-176°C, $[\alpha]^{22}_{D}$ = +41.3°, (c 0.92, CH_2Cl_2); HRFABMS (3-NBA/LiI as matrix), found, 831.4346 (calc. for $C_{42}H_{64}O_{16}Li$ 831.4354); EIMS 70 eV, m/z, 806[M-H_2O]$^+$ (19%), 788[806-H_2O^+] (100%), 774[806-CH_3OH]$^+$ (80%), 770[788-H_2O)]$^+$ (33%); FABMS, m/z, 831[M+Li]$^+$ for mol. wt. 824 corresponding to $C_{42}H_{64}O_{16}$; IR (thin film) ν_{max} cm^{-1}: 3460 (OH), 1730 (COO), 1650 (C=C), 1170 (COOR); NMR (^{1}H and ^{13}C) appear in Tables 2-4; and P388 ED_{50} = 0.33 μg/ml.

Acetylation of bryostatin 5 (5). To bryostatin 5 (5, 0.8 mg) in acetic anhydride (100 μl) was added 50 μl of pyridine. The reaction course was monitored by TLC. After 10 hr, reaction was complete and afforded 0.8 mg of bryostatin 5 26-acetate (14a); R_f=0.61 (in 5:4 hexane:ethyl acetate), 0.90 (in 3:1:1 toluene:ethyl acetate:methanol), 0.40 (in 9:1 methanol-water, RP C-8 plate).

Acetylation of bryostatin 14 (14) A sample (0.8 mg) of bryostatin 14 (14) was acetylated as summarized above for bryostatin 5 (5), except for a 20 hr reaction period. HPLC yielded 0.5 mg of pure bryostatin 14 20,26-diacetate identical (by TLC and 400 MHz ^{1}H-NMR) with bryostatin 5 26-diacetate and exhibiting $[\alpha]^{22}_{D}$ + 73.2° (c 0.59, CH_2Cl_2).

Bryostatin 15 (15). *Bugula neritina* (1,000 kg, damp wt) was recollected from the U.S. Southern California Coast in 1987. The animal was preserved in 2-propanol and this solution

was partitioned with methylene chloride to produce a fraction (6.13 kg) that was partitioned between hexane and 9:1 methanol-water. The hexane fraction was evaporated under reduced pressure to produce 4.3 kg of hexane extract. The methanol water portion was adjusted to a concentration of 3:2 and extracted with methylene chloride. Removal of solvent from the methylene chloride fraction afforded 1.25 kg of a P388 cell line active fraction, which was subjected to a series of steric exclusion and partition column chromatographic steps using Sephadex LH-20.[21] Typically, 40 to 45 g aliquots of active methylene chloride fraction were applied to Sephadex LH-20 in 1:1 methylene chloride-methanol. Fractions containing bryostatins 1 and 2 were located by TLC (95:5 methylene chloride-methanol) giving a combined weight of 348.7 g. Partition chromatography of 26 g aliquots on Sephadex LH-20 using hexane:toluene:methanol (3:1:1) provided separate fractions enriched in bryostatin 1 (37.51 g) and bryostatin 2 (14.11 g). High speed countercurrent distribution allowed further purification of bryostatins 1 and 2. A biphasic solvent system was prepared from hexane-ethyl acetate-methanol-water (4.5:1.5:1:0.3). HSCCD was accomplished with the Ito horizontal flow-through coil planet centrifuge using the planet gear drive at $\sim$ 800 rpm. The column consisted of 2.6 mm PTFE tubing (approx. volume 375 ml). The column was filled with the lower (stationary) phase of the two phase solvent system and counter-balanced. An 0.80 g aliquot (from 37.51 g of bryostatin 1 enriched fraction) dissolved in 3-4 ml of stationary phase was pumped into the column. The upper phase of the solvent system (the mobile phase) was pumped into the column from a tail to head direction with planetary motion of the column. An FMI lab pump maintained pressure at 25-30 psi. Fractions were collected and monitored by TLC. From 140 fractions (18 ml each) collected (2.5 L mobile phase) over a 5 hr period, fractions 50-120 contained bryostatin 1. The resulting bryostatin 1 fraction weighed 0.18 g. The HSCCD was repeated (45x) to give 4.4 g total of nearly pure bryostatin 1. Similar experimental procedures were followed for purification of the bryostatin 2 containing fraction. The solvent system was prepared using hexane-ethyl acetate-methanol-water (4:2.5:1.2:0.5). The lower phase was stationary. After HSCCD (14x) a combined fraction (3.36 g) enriched in bryostatin 2 was obtained. Subsequent flash column chromatography (silica gel) with 1:1 hexane-ethyl acetate for bryostatin 1 separation and 9:1 ethyl acetate-hexane for bryostatin 2 separation, and crystallization from ethyl acetate-hexane produced bryostatin 1 as an amorphous solid, mp 226-30°C (1.5 g, 1.5 x 10^{-4}% yield) and bryostatin 2, mp 186-187°C, (2.0 g, 2.0 x 10^{-4}% yield). The mother liquor from the bryostatin 1 crystallization was separated by HPLC with a Prepex 5-20 silica column (10 mm x 25 cm). Elution with hexane-methylene chloride-methanol (14:8:1) at a flow rate of 0.8 ml/min gave bryostatin 15 as an amorphous solid, mp 140-141°C (8.6 mg, 8.6 x 10^{-7}% yield) and bryostatin 8 (5.6 mg, 5.6 x 10^{-7}% yield). The known bryostatins 1, 2 and 8 were identified by direct comparison (principally by 400 MHz ^{1}H NMR and TLC) with authentic samples.

Bryostatin 15 exhibited FABMS m/z 927 $[M+Li]^+$ for mol. wt. 920 corresponding to $C_{47}H_{68}O_{18}$; $[\alpha]^{25}_D$ + 26° (c 0.27, CH_3OH); UV (CH_3OH) λ_{max} 227 nm (ϵ 25,995); IR (thin film) ν_{max} 3464, 2923, 2845, 1735, 1470, 1375, 1360, 1240, 1135, 1090 cm^{-1}. For the ^{1}H and ^{13}C NMR see Tables 2 and 3.

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