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Revised Structure of Bryostatin 3 and Isolation of the Bryostatin 3 26-Ketone from *Bugula neritina*

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Bryostatin 3 and the corresponding 26-ketone **5**, which represents a new bryostatin, were isolated from the marine bryozoan *Bugula neritina* (Bugulidae). Spectroscopic data from 2D NMR studies indicate that the structure of bryostatin 3 (**3**) has to be revised to structure **4**. Bryostatin 3 showed [^{3}H]phorbol dibutyrate receptor displacement activity similar to that of bryostatin 1, whereas the corresponding 26-ketone **5** was significantly less active.

Introduction

The bryostatins comprise a group of 15 macrocyclic lactones isolated from the marine bryozoan *Bugula neritina*.[2,3] Bryostatin 1 (**1**), the best studied member of this group, has interesting biological properties, e.g., it binds to and activates protein kinase C,[4-6] has strong cytostatic effects against the murine P388 lymphocytic leukemia in vitro and in vivo,[2] stimulates hematopoietic progenitor cells,[7] and has immunomodulating properties.[8] Recently, bryostatin 1 has been considered as a unique biological response modifier.[9] The structure of bryostatin 1 has been determined by X-ray analysis,[2] whereas the structures of bryostatins 2-13 have been determined by comparing NMR data with those obtained from bryostatin 1. Recently, the isovalerate ester side chain of bryostatins 4, 5, and 10[10-12] has been revised to pivalate.[13] Bryostatin 3 (**3**) shares the "bryopyran" macrocycle typical of bryostatins but differs by an additional lactone group on the C-19/C-23 pyran ring[14] (see Figure 1).

In the course of a large-scale isolation of bryostatin 1,[15] we have isolated bryostatin 3 and the corresponding 26-ketone **5**. Results from extensive 2D NMR studies presented in this report suggest that the structure of bryostatin 3 (**3**) has to be revised to **4**.

Results and Discussion

Freshly collected *Bugula neritina* (approx. 150 kg damp weight) was extracted with i-PrOH and MeOH. The combined extracts were partitioned between EtOAc and water and the EtOAc partition was fractionated on Florisil columns as described in the Experimental Section. Resulting fractions were further purified by combinations of reversed-phase flash chromatography, preparative HPLC on normal and reversed phases, Sephadex LH-20 chromatography, and high-speed countercurrent chromatography (HSCCC), yielding compound **4** (10 mg) and compound **5** (9 mg). Bryostatins 1 and 2 were also isolated from the same extract.

Compounds **4** and **5** showed chromatographic and spectroscopic properties similar to those of the bryostatins. Structures were elucidated by HR FABMS and 2D NMR spectroscopy (COSY, NOESY, HMQC, HMBC). NMR data of **4** and **5** were compared with recently revised ^{1}H and ^{13}C NMR assignments of bryostatin 1[16] and with data originally reported for bryostatin 3.[14]

The HR FAB mass spectrum of **4** (*m/z* 895.4331 [M + Li]$^+$) indicated an empirical formula of $C_{46}H_{64}O_{17}$ corresponding to 16 mass units less than bryostatin 1. ^{1}H and ^{13}C NMR chemical shifts of **4** are listed in Tables I and II, and results from homo- and heteronuclear correlation experiments (2D NMR) are summarized in Table III. Chemical shifts of **4** and observed homo- and heteronuclear correlations were consistent with a bryostatin 1 partial structure but showed differences for the C-19/C-23 pyran ring. The ^{1}H NMR spectrum of **4** was characterized by the presence of only one methoxy group (δ 3.71, 51.17, single cross peak in the corresponding HMQC spectrum), whereas other bryostatins show two methoxy functions (e.g., double cross peak in the HMQC spectrum of bryostatin 1 at δ 3.67, 3.70/51.04 (2×)).[16] Correlation peaks H-38/C-31 and H-30/C-31 in the HMBC spectrum of **4** indicated that the methoxy group was located on the C-11/C-15 pyran ring. Thus, structural differences between bryostatin 1 and **4** had to involve the C-19/C-23 pyran ring. The COSY spectrum of **4** (Figure 2a, Table III) revealed a spin system that included protons H-27, H-26, H-25, H-24a, H-24b, H-23, H-22, and H-34. Among these was a distinct doublet of doublets at δ 4.48. This resonance appears to be unique since it has not been found in the spectra of other bryostatins. This signal was assigned to the H-22 proton on the basis of HMBC correlations (op-

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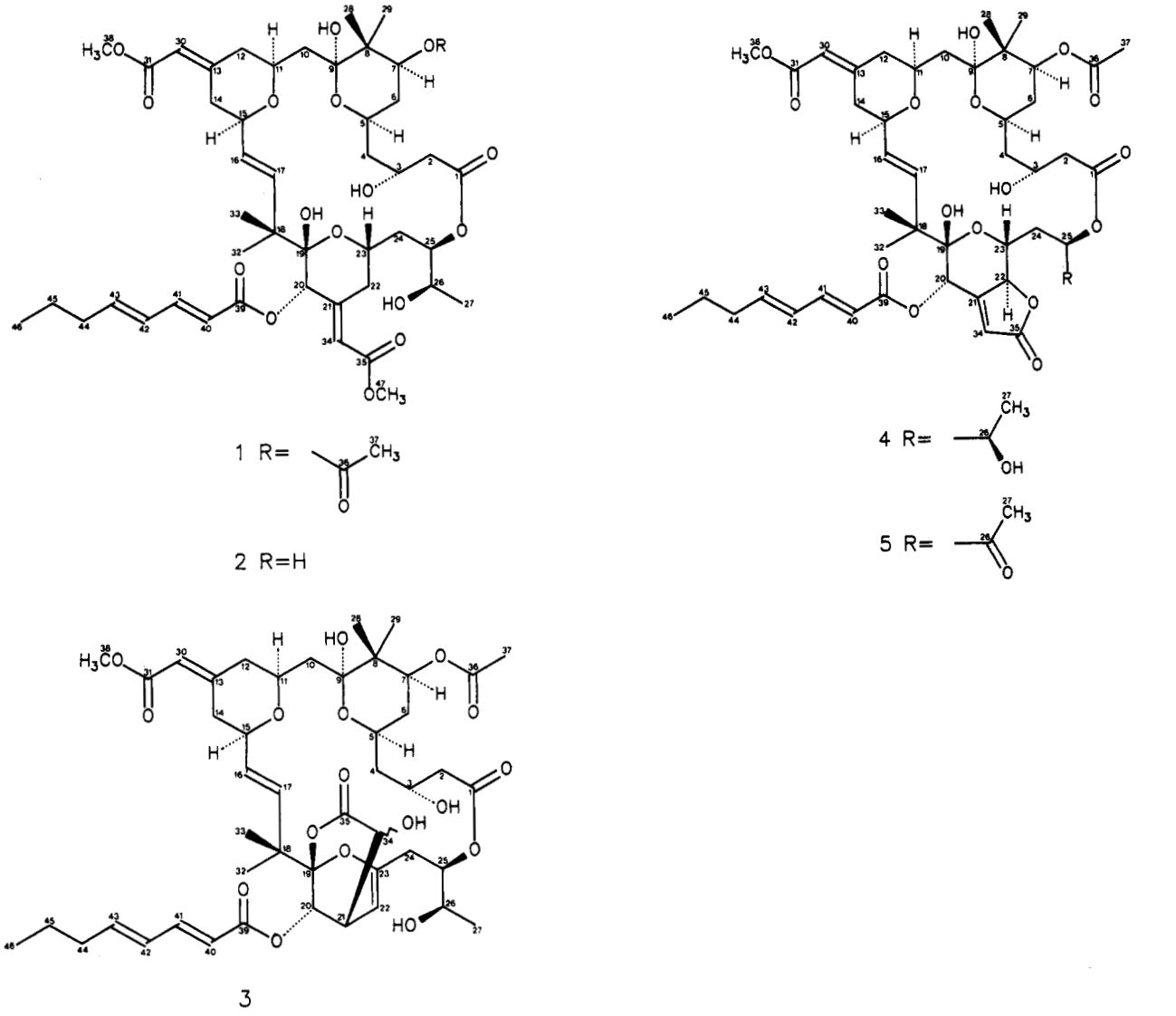

Figure 1. Structures of bryostatin 1 (**1**), bryostatin 2 (**2**), bryostatin 3 [**3** (old structure) and **4** (revised structure)], and bryostatin 3 26-ketone (**5**).

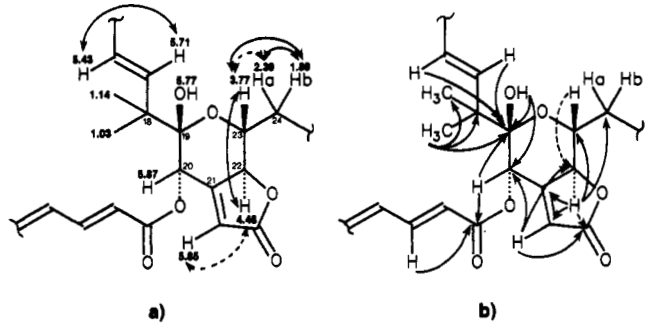

Figure 2. Bryostatin 3 revised partial structure (**4**): (a) COSY connectivities observed as strong cross peaks (solid lines) and as weak cross peaks (broken lines); (b) HMBC connectivities optimized for J = 8 Hz (solid lines) and optimized for J = 4 Hz (broken lines). Connectivities of second methyl group attached to C-18 omitted for clarity.

timized for $^{n}J_{CH}$ = 8 Hz) with C-21, C-23, C-24, and C-34 (Figure 2b). The chemical shift of the corresponding carbon (C-22, δ 81.23) suggested an oxygen function in that position, marking a major structural difference between **4** and bryostatin 1. The HMBC experiment was repeated with parameters optimized for $^{n}J_{CH}$ = 4 Hz and additional correlation peaks (H-23/C-22, H-22/C-35) confirming the C-22 assignment were observed. The quaternary carbon at δ 101.65 was unambiguously identified as C-19 by 5 correlations with protons two–four bonds away (Figure 2b). A D_2O exchangeable proton at δ 5.77 showed HMBC correlations with C-19 and C-20, indicating a free hydroxyl on C-19. This was supported by the observed nuclear Overhauser effect between the 19-O*H* and the H-16 proton. Further evidence for a free hydroxyl on C-19 was provided by partial D_2O exchange (^{13}C NMR). Hydroxyl-bearing carbon signals appeared as doublets (C-3, C-9, C-19) or as a broad singlet (C-26), as previously shown with bryostatin 1.[16]

Partially overlapping signals of four protons (H-20, H-34, H-40, 19-OH) appeared between 5.77 and 5.88 ppm. The H-20 proton correlated with C-19, C-21, and C-39 and thus confirmed the site of attachment (C-20) of the octadienoate ester side chain. Chemical shifts of C-21, C-22, C-34, and C-35 were in agreement with an α,β unsaturated γ-lactone fused onto the C-19/C-23 pyran ring. The observed coupling constant $^{HH}J_{22-23}$ of 9.0 Hz was in favor of a trans relationship between protons H-22 and H-23 and supported the relative stereochemistry shown in Figure 2. Based on the spectral evidence presented, we suggest the structure shown in Figure 1 for compound **4**.

The fact that compound **4** and bryostatin 3 (**3**) have the same molecular weight and similar chromatographic behavior[17] prompted us to compare the NMR data of the two

Table I. ^{13}C NMR Chemical Shifts of Bryostatin 1 (1), Bryostatin 3 [3 (Old Structure) and 4 (Revised Structure)], and Bryostatin 3 26-Ketone (5)

carbon no.	1[a,b]	3[c]	4[a]	5[a]
1	172.21	172.48	172.27	170.73
2	42.28	41.82[d]	41.82	41.28
3	68.44	68.30	68.28	67.95
4	39.87	39.48	39.46	39.21
5	65.71	65.58	65.62	65.86
6	33.35	33.24[e]	33.30	33.32
7	72.92	73.05[d]	72.97	72.73
8	41.00	41.14	41.13	41.14
9	101.85	101.90	101.82	101.81
10	41.95	41.82[d]	42.04	42.27
11	71.52	71.46	71.37	71.25
12	44.19	44.19	44.12	43.91
13	156.82	157.11	156.70	155.60
14	36.42	36.43	36.38	36.23
15	79.10	*f*	78.18	78.41
16	129.50	132.77	132.58	132.67
17	139.19	136.38	136.33	135.80
18	44.89	45.23	45.26	45.39
19	99.02	101.71	101.65	101.74
20	74.09	68.60	68.57	68.44
21	152.01	167.12	166.96	166.66
22	31.32	81.24	81.23	80.72
23	64.71	69.12	69.08	68.64
24	35.93	33.24[e]	33.19	33.08
25	73.68	73.05[d]	72.91	74.22
26	70.15	69.77	69.70	204.34
27	19.77	19.30	19.33	25.66
28	16.86	16.99	17.03	17.05
29	21.10	21.15[d]	21.17	21.08
30	114.00	114.38	114.22	114.78
31	166.72	166.89	166.74	166.59
32	24.64	24.50	24.51[g]	24.49[h]
33	19.77	*f*	21.33[g]	21.15[h]
34	119.57	114.12	114.31	114.62
35	167.00	171.96	171.83	171.54
36	171.00	171.18	170.95	170.57
37	21.10	21.15[d]	21.17	21.21
38	51.04	51.18	51.17	51.04
39	165.58	165.98	165.87	165.83
40	118.63	117.37	117.28	117.21
41	146.35	147.72	147.62	147.74
42	128.39	128.38	128.28	128.29
43	145.44	146.65	146.58	146.66
44	35.04	35.13[d]	35.14	35.14
45	21.86	21.87	21.91	21.91
46	13.65	13.68	13.73	13.70
47	51.04			

[a] $CDCl_3$, 125 MHz. [b] Bryostatin 1 data.[16] [c] $CDCl_3$, 100 MHz. Unassigned chemical shifts from ref 14 were tentatively assigned on the basis of comparison to bryostatin (1) and bryostatin 3 (4) from this study. [d,e] Chemical shifts listed 2× (*d*) or 3× (*e*).[14] [f] No corresponding chemical shift. However, two extra carbon lines were listed for δ 35.13 (*d*) and 33.24 (*e*) in ref 14. [g,h] Assignments may be interchanged.

compounds more thoroughly (see Tables I and II). It is noteworthy that no ^{13}C NMR assignments were made in the original paper describing bryostatin 3 and that no 2D-NMR techniques were used.[14] We found the carbon-13 chemical shifts for 4 to be identical with those reported for 3, with the exception of shifts assigned to C-10, C-15, and C-33. In our study, these resonances were observed at δ 42.04, 78.18, and 21.30, respectively, whereas in the original report they were accounted for by listing other resonances 2–3 times (see footnote of Table I). The unusual chemical shift at δ 81.23, which we assigned to the C-22 carbon and which had also been reported[14] in the original listing of carbon chemical shifts (δ 81.24), indicated a substitution with oxygen. The corresponding proton signal H-22 (as determined by HMQC) appeared at δ 4.48 and did not exchange with D_2O. An equivalent signal (δ 4.47) was erroneously assigned[14] to the C-34 hydroxyl proton. Evidence from NMR data strongly indicates that compounds 3 and 4 are identical. This is supported by HR FABMS, IR, and UV data and by the fact that the compounds were isolated from the same organism collected in the same geographical area (Southern California coast). Consequently, the structure of bryostatin 3 has to be revised to 4.

Compound 5 ($C_{46}H_{62}O_{17}$ by HR FABMS) displayed NMR spectra similar to those of 4. Assignments are listed in Tables I and II and were confirmed by COSY, HMQC, and HMBC experiments (data not shown). The only structural difference between 4 and 5 was noted in the C-25 side chain. The signal of the C-26 carbon at δ 69.70 (4) was replaced with a carbonyl resonance at δ 204.34. The C-27 methyl protons appeared as a singlet at δ 2.18 compared to a doublet at δ 1.23 in the ^1H NMR spectrum of 4. The position of the carbonyl was determined as C-26 on the basis of HMBC correlations with the H-25 proton at δ 5.17 and the C-27 methyl protons. This partial structure was in agreement with HR FABMS data and thus structure 5 was assigned to the new compound, designated bryostatin 3 26-ketone. The compound may represent an artifact of the extraction/isolation process.

Binding of bryostatin 3 (4) and the corresponding 26-ketone 5 to the phorbol ester receptor was studied by using the [^3H]phorbol dibutyrate (PDBu) displacement assay described by De Vries et al.[5] Concentrations resulting in a 50% displacement of [^3H]PDBu (IC_{50}) were determined as follows: bryostatin 1 (0.8 ng/mL), bryostatin 2 (2.5 ng/mL), bryostatin 3 (6.6 ng/mL), and bryostatin 3 26-ketone (29 ng/mL). In an additional experiment, an artificial mixture of bryostatins 1, 2, 3, and 3 26-ketone was separated by HPLC and microfractions were collected and directly assayed for [^3H]PDBu displacement.[18] Percent displacements were plotted against the retention time and thus a "displacement chromatogram" was generated (Figure 3). Given the instability of 4 and 5, this method may give a more accurate picture of the relative bioactivities of these compounds.

PDBu displacement activity of bryostatin 3 (4) was similar to that of bryostatins 1 and 2. The original structure for bryostatin 3 (both 34-OH epimers) and the candidate structure were energy minimized to explore structure–activity relationships. It is interesting to note that our proposed structure 4 gave a lower relative energy than structure 3 by 15 kcal/mol, although low energy does not a priori justify a structure as correct.

The carbon skeleton of energy-minimized structures of bryostatin 1 and our proposed structure for bryostatin 3 are compared in Figure 4. Apart from the obvious differences in the five-membered lactone region, the overall shape of the molecule is preserved between the two bioactive bryostatins. The model of original structure 3 (not shown) diverged substantially from that of bryostatin 1. Previous SAR comparisons of the bryostatins[19] may need to be reviewed in light of the new structure. One prediction made by the model is that the 19-OH should be hydrogen bonded in transannular fashion as donor to 3-OH. An energy map calculation of potential 19-OH rotation predicts a low energy distance of 2.82 Å from

(17) An authentic sample of bryostatin 3 was not available, but see Figure 3 in this report and Figure 1 in ref 13 for a comparison.

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Table II. ^{1}H NMR Chemical Shifts of Bryostatin 3 [3 (Old Structure) and 4 (Revised Structure)] and Bryostatin 3 26-Ketone (5)

	3[a]	4			5		
proton no.	δ	δ	mult	J (Hz)	δ	mult	J (Hz)
2a	2.55	2.56	t	12.2	2.55	m	
2b	2.43	2.45	dd	12.3, 2.8	2.55	m	
3	4.03	4.05	m		4.11	m	
3-O*H*	4.34	4.32[b]	d	11.5	4.31	d	12.0
4a	1.9	2.01		m	1.98	m	
4b	1.55	1.58	ddd	14.8, 3.9, 3.1	1.58	ddd	15.1, 3.2, 3.2
5	4.17	4.21	tt	11.6, 2.3	4.21	tt	11.5, ~2.0
6ax	1.4	1.50	q	12.0	1.44	q	12.0
6eq	1.72	1.74	ddd	12.2, 4.5, 2.5	1.72	ddd	12.5, 4.7, 2.2
7	5.16	5.17	dd	11.7, 4.8	5.11	dd	11.8, 4.3
9-O*H*	nd[c]	nd			nd		
10a	nd	2.06	m		2.08	m	
10b	nd	1.67	d	15.0	1.63	d	15.1
11	3.98	3.98	ddd	11.2, 7.5, 2.3	3.80	m	
12a	2.17	2.18	m		2.15	m	
12b	2.17	2.08	m		2.05	m	
14ax	2.0	1.87	m		1.83	dd	12.5, 11.2
14eq	2.0	3.68	br d	~13.5	3.62	br d	~13.5
15	4.17	4.15	ddd	11.3, 8.5, 2.7	3.84	m	
16	5.41	5.43	dd	15.8, 8.3	5.39	dd	16.0, 8.1
17	5.70	5.71	d	15.7	5.57	d	15.8
19-O*H*	nd	5.77[b]	d	1.8	5.65	nd	
20	5.28	5.87	br d	1.9	5.87	br d	<2.0
21	1.85						
22	5.82	4.48	dd	9.0, 1.7	4.49	dd	9.1, 1.4
23		3.77	ddd	11.4, 9.0, 2.4	3.82	m	
24a	2.35	2.39	ddd	14.3, 12.3, 2.3	2.27	m	
24b	1.86	1.89	m		2.15	m	
25	5.02	5.06	ddd	12.4, 5.5, 3.0	5.17	dd	12.8, 3.2
26	3.78	3.82	dq	6.5, 5.8			
26-O*H*	nd	3.68[b]	nd				
27	1.21	1.23	d	6.5	2.18	s	
28	1.12	1.02	s		0.98	s	
29	1.00	0.95	s		0.90	s	
30	5.67	5.70	t	1.7	5.64	dd	2.5, 1.7
32	1.00	1.03	s		0.99	s	
33	0.92	1.14	s		1.12	s	
34	3.71	5.85	dd	1.8, 1.8	5.83	dd	1.7, 1.7
34-O*H*	4.47						
37	2.05	2.07	s		2.01	s	
38	3.69	3.71	s		3.65	s	
40	5.87	5.88	d	15.5	5.85	d	15.5
41	7.37	7.39	dd	15.5, 10.0	7.35	dd	15.3, 9.9
42	6.20	6.20	dd	15.1, 9.3	6.20	m	
43	6.20	6.25	dt	15.0, 6.3	6.21	m	
44	2.17	2.18	m		2.16	m	
45	1.47	1.49	sext	7.5	1.43	sext	7.4
46	0.92	0.94	t	7.5	0.89	t	7.4

[a] Bryostatin 3 assignments.[14] [b] Signal disappearing after D_2O exchange. [c] nd = not detected.

19-O*H* to 16-H, and an NOE effect is observed between these two protons.

In summary, spectroscopic data presented in this report clearly indicate that the structure of bryostatin 3 has to be revised to 4. This is supported by MM results and SAR data of bryostatins binding to the phorbol receptor.

Experimental Section

General. Solvents for extraction were technical grade; other solvents were HPLC grade (Burdick & Jackson). Diatomaceous earth, Dicalite Speed Flow, was obtained from Grefco Inc. and Florisil 100/200 Å from US Silica Company, Berkeley Springs, WV, respectively. Sephadex LH-20 was purchased from Pharmacia LKB Inc. Preparative HPLC separations were carried out on normal- and reversed-phase Dynamax 60 Å 8-μm columns (250 × 21.4 or 41.4 mm i.d.) equipped with corresponding 50-mm precolumns (Rainin Instr., Woburn, MA), using a Delta Prep 3000 system (Waters) and a Model 153 UV (254 nm) detector (Beckman). Analytical HPLC was performed on Microsorb C-18, 3 μm, 100 × 4.6 mm i.d. columns (Rainin).[18] The Ito multilayer coil planet centrifuge for HSCCC, equipped with 2.6-mm i.d. tubing, was obtained from P.C. Inc., Potomac, MD. NMR experiments were performed on a Varian VXR500S spectrometer using equipment and methods as described previously.[16] Spectra were recorded in $CDCl_3$ and chemical shifts are given in ppm relative to TMS (^{1}H NMR) and relative to the solvent shift ($CDCl_3$ at 77.0 ppm) for ^{13}C NMR spectra. FAB mass spectra were obtained in a dithiothreitol matrix using a VG Micromass ZAB-2F instrument. IR spectra were recorded on a Perkin-Elmer 1430 ratio recording infrared spectrophotometer. UV spectra were recorded online during analytical reversed-phase HPLC analysis of the samples with photodiode array detection. Molar absorptivities are not reported due to the instability of both **4** and **5**. The general shape of the curve and relative molar absorptivities at both λ_{max} for **4** and **5** parallel that of bryostatin 1. The combined HPLC/[^{3}H]phorbol dibutyrate displacement assay was performed by separating a mixture of bryostatins 1, 2, 3, and 3 26-ketone (200 ng each/injection) by reversed-phase HPLC (see above). Elution was carried out with aqueous MeCN 78% at 1 mL/min; fractions were collected in assay tubes every 3–4 s and directly analyzed.[18] IC_{50} values are averages of three experiments. The Cambridge Crystallographic Database X-ray crystal structure for bryostatin 1 was used as a starting point for the modeling studies. Molecular mechanics was run using the program MacroModel, version 2.5, using the MM2 parameter set without modification.[20]

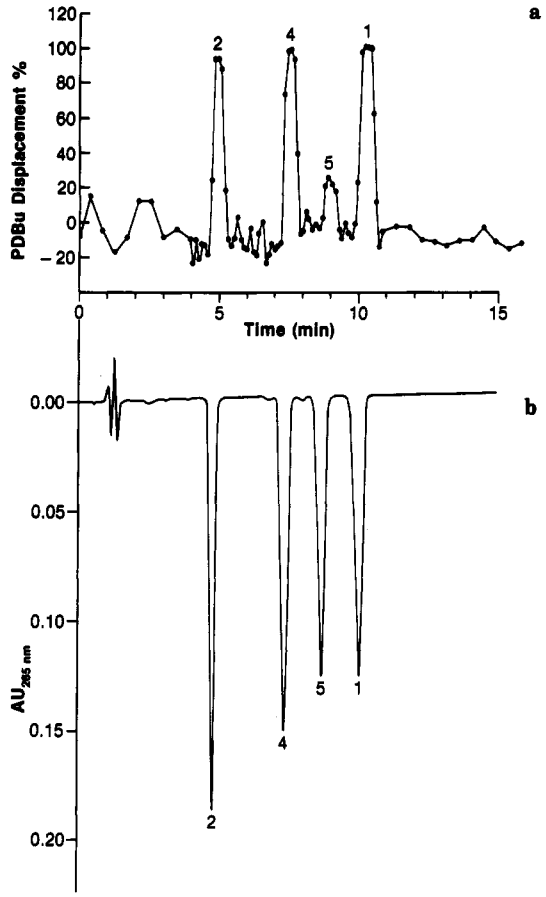

Figure 3. Combined HPLC/[^{3}H]phorbol dibutyrate (PDBu) displacement assay: (a) "chromatogram" generated with displacement values measured for microfractions collected from HPLC; (b) HPLC chromatogram (see Experimental Section for conditions) for compounds (200 ng, each) bryostatin 1 (1), bryostatin 2 (2), bryostatin 3 (4), and bryostatin 3 26-ketone (5).

Graphical output was produced by downloading structure files in the Chem 3D format to Macintosh and printed by using the program Chem 3D Plus, version 2.0.1.

Extraction and Isolation. *Bugula neritina* was collected during the spring of 1988 near Long Beach, CA, at depths averaging 25–30 ft (Marinus Inc., Long Beach, CA). Animals were packed in two 55 gallon drums and covered with i-PrOH. After shipment, the i-PrOH was removed and the material was further extracted with MeOH. The organic extracts were combined and solvents were evaporated under reduced pressure. The resulting extract was further partitioned between EtOAc and water. The organic phase was separated and evaporated to dryness, yielding 480 g of crude extract. Aliquots (2 × 150 g) of this extract were coated on dicalite (1.5 kg) and loaded onto a Florisil column. Elution was carried out with solvents of increasing polarity, affording hexane (9.2 g), CH_2Cl_2 (96.0 g), EtOAc (55.5 g), and MeOH fractions. An aliquot of the EtOAc fraction (30 g) was coated on dicalite (200 g) and loaded onto a Florisil column (10 × 8 cm i.d.) for flash chromatography. Elution was carried out with hexane–acetone mixtures (90:10, 3.0 L), (80:20, 4.5 L), and (70:30, 4.5 L). Fractions containing 4 and bryostatin 1 (elution volume 2.0–7.5 L) were combined and evaporated to dryness. The residue (6.07 g) was coated on reversed-phase material (70 g) obtained from a Waters PrepPAK 500/C18 cartridge (55–105 μm) and purified by flash chromatography on the same material (8 × 8 cm, MeOH–H_2O 90:10). The resulting fraction (2.28 g, elution volume 400–1200 mL) was separated by preparative HPLC (C-8, 41.4 mm i.d.; sample load, 0.5–1.0 g) using MeCN–H_2O (85:15) as the mobile phase. The fraction containing 4 was rechromatographed under the same conditions in MeCN–H_2O (80:20), affording crude 4 (22 mg). This material was further purified on Sephadex LH-20 (30 × 2 cm i.d., CH_2Cl_2–hexane 3:1). The resulting fraction (13 mg) was finally purified by preparative HPLC

Table III. Homo- and Heteronuclear Correlation (2D NMR) of Bryostatin 3 (4)[a]

proton no.	COSY [NOESY]	HMQC	HMBC[b]
2a	H-2b, H-3	C-2	C-1, C-3, (C-4), C-25[c]
2b	H-2a	C-2	C-1, (C-3)
3	H-2a, 3-OH	C-3	nd[f]
3-O*H*	H-3		nd
4a	H-4b, H-5	C-4	C-3
4b	H-3, H-4a, (H-5)[g]	C-4	nd
5	H-4a, (H-4b), [H-7] (H-6eq), H-6ax	C-5	nd
6ax	H-5, H-6eq, H-7	C-6	C-5, C-7, (C-8)
6eq	(H-5), H-6ax, H-7	C-6	(C-5), C-7, C-8
7	H-6ax, H-6eq, [H-5, H-29]	C-7	C-6,[c] C-8, C-28, C-29, C-36
9-O*H*	nd		C-9[d]
10a	H-10b, H-11	C-10	C-8, C-9, C-11
10b	H-10a	C-10	C-5,[c] C-8, C-12
11	H-10a, H-12a	C-11	C-9[d]
12a	H-11, H-12b, (H-30)	C-12	C-10, C-11, C-13, C-30
12b	H-12a, (H-14eq)	C-12	C-11
14ax	H-14eq, H-15, (H-30)	C-14	C-15, (C-16), C-30
14eq	H-12b, H-14ax, (H-15)	C-14	C-12, C-13
15	(H-14eq), H-14ax, H-16	C-15	C-16[c], C-17[c]
16	H-15, H-17, [19-OH]	C-16	C-14,[c] C-15, C-19, C-33[c]
17	H-16	C-17	C-15,[c] C-19, C-32, C-33
19-O*H*	nd, [H-16]		C-19, C-20
20	nd	C-20	C-19, C-21, C-39[e]
22	H-23, (H-34)	C-22	C-21, C-23, C-24, (C-34), (C-35)[c]
23	H-22, (H-24a), H-24b	C-23	C-22[c]
24a	(H-23), H-24b, H-25	C-24	C-25, C-26
24b	H-23, H-24a, (H-25)	C-24	nd
25	H-24a, (H-24b), H-26	C-25	C-1,[c] C-26[c]
26	H-25, H-27	C-26	(C-24), (C-25), C-27
26-O*H*	nd		nd
27	H-26	C-27	C-25, C-26
28	nd	C-28	C-7, C-8, C-9, C-29
29	nd, [H-7]	C-29	C-7, C-8, C-9, C-28
30	(H-12a), (H-14ax)	C-30	C-12, C-14, C-31
32	nd	C-32	(C-17), C-18, C-33
33	nd	C-33	C-17, C-18, C-19, C-32
34	(H-22)	C-34	(C-20), C-22, C-35
37	nd	C-37	C-36
38	nd	C-38	C-31
40	H-41	C-40	C-39,[e] C-42
41	H-40, H-42	C-41	C-39, C-40,[c] C-42,[c] C-43
42	H-41, H-43	C-42	C-40,[c] C-41, C-44
43	H-42, H-44	C-43	C-41, C-42,[c] C-44
44	H-43, H-45	C-44	C-42, C-43, C-45, C-46
45	H-44, H-46	C-45	C-43, C-44, C-46
46	H-45	C-46	C-44, C-45

[a] Solvent $CDCl_3$. [b] Connectivities optimized for $^nJ_{CH}$ = 8 Hz. [c] Cross peak only detected when connectivities were optimized for $^nJ_{CH}$ = 4 Hz. [d] Observed signal could be H-11/C-9 and/or 9-O*H*/C-9 cross peak. [e] H-20/C-39 and H-40/C-39 cross peaks overlapping; cross peaks resolved when connectivities optimized for $^nJ_{CH}$ = 4 Hz. [f] nd = not detected. [g] Parentheses indicate a cross peak of low relative intensity.

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(21) **Note Added in Proof.** Concurrently, Pettit et al. (*J. Org. Chem.* **1991**, *56*, 1337) reported on the structure revision of bryostatin 3. The revised structure is identical with structure 4 in our report; however, the structure reported in Pettit's paper shows the opposite stereochemistry at C-22.

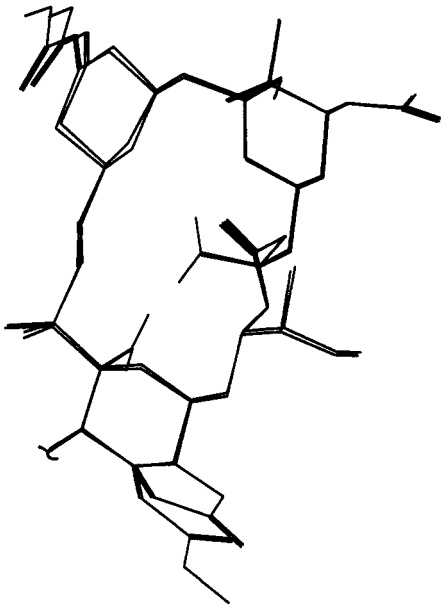

Figure 4. Superimposed energy minimized models (MM2) of bryostatin 1 and bryostatin 3 (C-20 ester side chain omitted for clarity).

on silica gel (21.4 mm i.d. column, hexane–acetone (7:3) as mobile phase), affording pure **4** (10 mg): IR (KBr) 3450, 2970–2920, 1785, 1735, 1715, 1650–1640, 1365, 1305, 1275, 1245, 1165, 1145, 1095, 1070–1040, 980 cm^{-1}; HR FABMS m/z 895.4331 $[M + Li]^+$, calcd for $C_{46}H_{64}O_{17}$ Li 895.4304, Δ = 3 ppm; UV (70% aqueous CH_3CN) λ_{max} (nm) 230, 266.

A second aliquot (25 g) of the EtOAc fraction was purified on Florisil (see above; solvent, hexane–acetone 90:10, 85:15, 80:20, and 70:30, 2.0 L each mixture). Fractions containing **5** (elution volume 5.0–7.0 L; 2.17 g) were combined and further separated on reversed-phase material (sample was coated on 40 g of packing obtained from a Waters PrepPAK 500/C18 cartridge, 55–100 μm, column 6 × 8 cm i.d.; MeOH–H_2O 90:10, elution volume 750–1050 mL), affording 418 mg. This fraction was purified by preparative HPLC (C-8, 21.4 mm i.d., MeCN–H_2O 88:12, sample load 40–80 mg) and 48 mg of crude **5** was obtained. The compound was finally purified by HSCCC in the solvent system hexane–EtOAc–MeOH–H_2O (14:6:10:7) (sample in 1:1 mixture (7 mL) of upper phase and lower phase; mobile phase = upper phase; flow of 5 mL/min; fractions 5 mL each; retention of stationary phase was ca. 90%). Pure **5** was obtained in fractions 28–42 (9 mg): IR (KBr) 3440, 2935–2920, 1780, 1740, 1720, 1650–1640, 1365, 1305, 1275, 1245, 1165, 1145, 1130, 1095, 1075, 1045, 1025, 1000, 980 cm^{-1}; HR FABMS m/z 893.4156 $[M + Li]^+$, calcd for $C_{46}H_{62}O_{17}$ Li 893.4147, Δ = 0.1 ppm; UV (70% aqueous CH_3CN) λ_{max} (nm) 228, 266.

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On the Remarkable Propensity for Carbon–Carbon Bond Cleavage Reactions in the C_8–C_{10} Region of FK-506

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It has been deduced from a series of transformations that formation of a tetrahedral intermediate at C_9 in FK-506 occasions fragmentation of the C_9–C_{10} bond by a retro-Claisen-like pathway or the C_9–C_8 bond by a benzilic acid type rearrangement. Reduction of FK-506 with L-Selectride leads to the formation of a boronate ester **18** rather than to the corresponding diol **17**, which had previously been formulated. Direct reduction of FK-506 with sodium triacetoxyborohydride (or hydrolysis of **18**) does provide access to (22*S*)-dihydro FK-506 **17**. The former reduction also leads to some 22*R* epimer, which is an intermediate in the total synthesis of FK-506.

Background

The enormous immunosuppressive activity of FK-506 (**1**)[1] has served to foster renewed interest in the use of organic molecules, of a nonpeptidyl nature, as modulators of the human immune system.[2] Particularly exciting is the fact that the potency of FK-506 is ca. 100 times greater than cyclosporin, which is the benchmark compound in the field.[3] While there have been some dramatic claims on behalf of FK-506 in suppressing rejection of various human organ transplants,[4] its generality as an adjutant for transplantation surgery has not yet been conclusively demonstrated.

Although a number of synthesis-based investigations in the FK-506 area have been described,[5] very few studies

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