Journal of Natural Products
Vol. 49, No. 4, pp. 661-664, Jul-Aug 1986

ANTINEOPLASTIC AGENTS, 118. ISOLATION AND STRUCTURE OF BRYOSTATIN 9[1]

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Marine Bryozoa in the Bugulidae family are generally very efficient at establishing new colonies (usually flexible) that may reach 5 cm in length. Part of this success has been attributed to the snapping beak (avicularia) protecting each zooid (2). While well known for its colonizing success,[2] *Bugula neritina* L., unlike other closely related *Bugula* species, does not have avicularia. Results of our chemical investigation of this fascinating animal suggest that its notable survival abilities may reside in extraordinary chemical rather than physical defenses.

In addition to the new class of antineoplastic constituents, such as bryostatin 4 (**1a**) (3), which we isolated from *B. neritina*, careful separation of a sterol fraction has led to isolation of dinosterol (4) and the new 3β-hydroxy-23*S*,24*R*-dimethyl-5α-cholestane (5) that represent important pathways of dinoflagellate sterol biosynthesis. Thus, *B. neritina* may serve as host for zooxanthellae-type dinoflagellates and use the symbiotic association as a source of potent defensive and/or offensive (6,7) substances. What role, if any, such zooxanthellae might have in biosynthesis of the bryostatins or their precursors is still unknown. But we have now succeeded in uncovering a new bryostatin designated 9 (**2**) that significantly inhibits (PS $ED_{50}=1.2\times10^{-3}$ μg/ml with 40% life extension at 80 μg/kg) growth of the U.S. National Cancer Institute's P-388 lymphocytic leukemia (PS system), and a summary of that study follows.

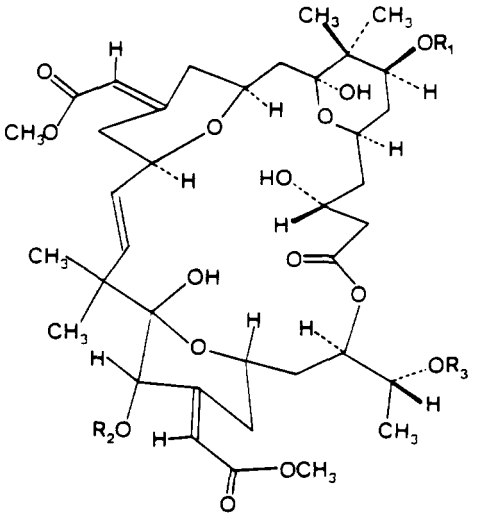
In depth examination of *B. neritina* PS active fractions that led to bryostatin 4 (3) later provided bryostatins 5-7 (e.g., **1b**, 8). Later, bryostatin 8 (**1c**) was isolated from the marine bryozoan *Amathia convoluta* (6). Since earlier investigation (9) of such *B. neritina* fractions revealed the possible presence of some seventeen antineoplastic bryostatins, we have continued attempts to isolate the remaining active components. In the present study, 50 kg (wet wt) of a 1982 Gulf of Mexico (Florida)[3] re-collection of *B. neritina* was treated as described for obtaining bryostatin 4 (3), and this led to isolation of the closely related bryostatin 9 (**2**, 13.5 mg, 2.7×10^{-5}% yield).

The structure of the new bryostatin was determined by a detailed analysis of the 400 MHz ^{1}H nmr (see structure **2**), and solution phase secondary ion mass (sp-sims, 10) spectral data. The 400 MHz ^{1}H-nmr spectrum of bryostatin 9 (**2**) was found to be very similar to that of bryostatin 6 (**1b**). Also, the mass spectrum of bryostatin 9 gave ions at *m/z* 875 $[M+Na]^+$, [M^+, 852 for $C_{43}H_{64}O_{17}$], 815 $[M+Na-60]^+$, and 787 $[M+Na-88]^+$ as previously found for bryostatin 6 ($C_{43}H_{64}O_{17}$, butyrate, and acetate esters at C-7 and C-20) and again suggested the presence of both acetate and butyrate esters. Thin layer chromatographic analysis of bryostatins 6 (**1b**) and 9 (**2**) showed analogous, but not identical, mobilities. The corresponding acetates **1d** and **1e**, prepared by acetylation with Ac_2O-pyridine, were also different. Therefore, the ace-

[1]For Part 117, see Pettit *et al.* (1).

[2]*B. neritina* is especially recognized as a worldwide ship-fouling organism.

[3]The presence of bryostatin 9 was also detected as a mixture with bryostatin 11 in fractions from a Gulf of California (Sonora, Mexico) re-collection.

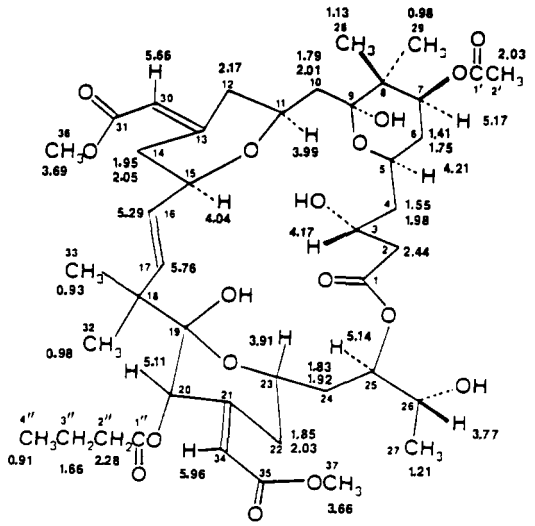


1a R_1=$COCH_2CH(CH_3)_2$, R_2=$COCH_2CH_2CH_3$, R_3=H (Bryostatin 4)
1b R_1=$COCH_2CH_2CH_3$, R_2=$COCH_3$, R_3=H (Bryostatin 6)
1c R_1=R_2=$COCH_2CH_2CH_3$, R_3=H (Bryostatin 8)
1d R_1=$COCH_2CH_2CH_3$, R_2=$COCH_3$, R_3=$COCH_3$
1e R_1=$COCH_3$, R_2=$COCH_2CH_2CH_3$, R_3=$COCH_3$
1f R_1=H, R_2=$COCH_2CH_2CH_3$, R_3=H

tate and butyrate substituents of bryostatin 9 appeared to be reversed at positions 7 and 20 from those of bryostatin 6.

To verify the tentative structural assignment, bryostatin 9 (**2**) was treated with 1% HCl in MeOH at ambient temperature for 3 days. The crude product was purified using hplc (reversed phase C-18 chromatography) to yield (1 mg→0.65 mg) the major product (**1f**). The hydrolysis product was found to be identical with the principal acid-catalyzed cleavage product from bryostatin 8 (**1c**). From this evidence combined with a spectral analysis, the 7-hydroxy 20-butyrate structure **1f** was assigned to the bryostatin 9 hydrolysis product and thereby structure **2** to bryostatin 9.

Interestingly, the reversal of ester groups in bryostatins 6 (PS ED_{50} 1.0×10^{-5} μg/ml and T/C 139-182 at



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46↔185 μg/kg) and 9 did not appear to affect strongly the PS activity. Present evidence suggests that additional (and important) structure/activity information will be obtained by isolating other new antineoplastic substances produced by the versatile *B. neritina*.

EXPERIMENTAL

Introduction to the experimental section of Pettit *et al.* (3) provides a summary of chromatographic and instrumental techniques employed in the present investigation. The same reference includes the animal taxonomy and re-collection data. In summary, *B. neritina* (50 kg wet wt) from a 1982 Gulf of Mexico (Florida) re-collection served as starting material for this study. The initial CH_2Cl_2 fraction from a CH_2Cl_2/MeOH extraction procedure was subjected to a 9:1↔4:1 MeOH-H_2O solvent partitioning sequence with hexane↔CCl_4. The latter chlorocarbon fraction was extensively separated (PS bioassay guidance) as described by Pettit *et al.* (6), using gel permeation and partition chromatography on Sephadex LH-20, silica gel, and reversed phase column chromatography, preparative layer and high performance liquid chromatography. The fraction that yielded bryostatin 4 (3) led to bryostatin 9.

ISOLATION OF BRYOSTATIN 9 (**2**).—Bryostatin 9 was isolated as colorless needles, melting at 159-162°, $[\alpha]^{28}D$ + 87.31 (c=0.04, MeOH); uv λ max (MeOH) 229 nm (ε 36,200); ir ν max (KBr) 3465, 3440, 2975-2940, 1735, 1725, 1655-1645, 1440, 1380, 1365, 1290, 1240, 1160, 1100, 1080, 1070, 1045, 1000, 870 cm^{-1}; sp-sims *m/z* 875 $[M+Na]^+$, 857 $[M+Na-18]^+$, 843 $[M+Na-32]^+$, 833 $[M+Na-42]^+$, 817 $[M+Na-58]^+$, 815 $[M+Na-60]^+$, 787 $[M+Na-88]^+$.

ACETYLATION OF BRYOSTATIN 9 (**2**).—Bryostatin 9 (1.2 mg) was acetylated with Ac_2O-(0.1 ml) and pyridine (0.15 ml for 4 h at room temperature. The mixture was concentrated under N_2 gas and dried. The hplc reversed phase column (C-18) chromatography of the crude product with MeOH-H_2O mixture (from 50:50 to 90:10) gave 0.7 mg of the acetate (**1e**), from CH_2Cl_2/MeOH mixture, as colorless needles melting at 155-159°, $[\alpha]^{27}D$, +95.70 (c=0.05, MeOH); uv λ max (MeOH) 228 nm (ε 35,500); ir ν max (KBr) 3465, 2980-2950, 1740, 1725, 1655-1640, 1438, 1375, 1365, 1285, 1240, 1160, 1100, 1085, 1070, 1048, 1000, 875 cm^{-1}; sp-sims *m/z* 917 $[M+Na]^+$, (M^+, 894 for $C_{45}H_{66}O_8$), 899 $[M+Na-18]^+$, 855 $[M+Na-32]^+$, 875 $[M+Na-42]^+$, 873 $[M+Na-44]^+$, 871 $[M+Na-46]^+$, 859 $[M+Na-58]^+$, 857 $[M+Na-60]^+$, 829 $[M+Na-88]^+$; tlc Rf 0.31 [Rf of bryostatin 6 acetate (**1d**) 0.37 with n-hexane-Me_2CO (7:3, silica gel)].

HYDROLYSIS OF BRYOSTATIN 9 (**2**).—A 1.0 mg sample of bryostatin 9 (**2**) was hydrolyzed for 3 days at 18-20° in 0.4 ml of 1% HCl in MeOH. The mixture was concentrated under N_2 gas and dried. By hplc reversed phase column (C-18) chromatography using a MeOH/H_2O gradient, the hydrolysis product (**1f**) was isolated in 0.65 mg yield. The compound (**1f**) was obtained as a colorless powder, melting at 147-149°, $[\alpha]^{26}D$ +87.24 (c=0.03, MeOH); uv λ max 229 nm (ε 36,100); ir λ max (KBr) 3470, 2980-2950, 1742, 1725, 1658-1640, 1435, 1380, 1370, 1288, 1240, 1160, 1100, 1090, 1075, 1050, 1000, 870 cm^{-1}; sp-sims *m/z* 833 $[M+Na]^+$ (M^+, 810 for $C_{41}H_{62}O_{16}$), 815 $[M+Na-18]^+$, 775 $[M+Na-58]^+$, 745 $[M+Na-88]^+$; tlc Rf 0.45 on a reversed phase tlc plate with MeOH-H_2O (4:1). Degradation product **1f** gave the same physical properties as found for the hydrolysis product obtained from bryostatin 8 (**1c**) (6).

ACKNOWLEDGMENTS

Financial support very necessary to this research was provided by Eleanor W. Libby, the Waddell Foundation (Donald Ware), Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), the Fannie E. Rippel Foundation, the Arizona Disease Control Research Commission, the Flinn Foundation, the Robert B. Dalton Endowment Fund, the Upjohn Co., Virginia L. Bayless, Grants CA 16049-07-09 awarded by the NCI, DHHS and Contract NO1-CM-97262 with the Division of Cancer Treatment, NCI, DHHS. For other assistance we thank the Smithsonian Institution Oceanographic Sorting Center, the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723), and Drs. H. Cohen, D.L. Doubek, P.D. Ellis, G. Hendler, M.I. Suffness, J.M. Schmidt, and Mr. Phillip J. Daschner and Mr. J.J. Rudloe.

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Received 7 October 1985