boration–carbenoidation–oxidation reaction. The chiral auxiliary, α-pinene, can be readily recovered and recycled, making the asymmetric synthesis exceptionally efficient. With the increasing knowledge of organoboranes, the asymmetric synthesis of chiral products via carbon–carbon bond formation has now become more attractive. We are continuing to explore asymmetric synthesis via chiral organoboranes.

(18) The Varian XL-200 spectrometer was purchased with funds from NSF Grant CHE-8004246. This support is gratefully acknowledged.

Isolation and Structure of Bryostatin 1[1]

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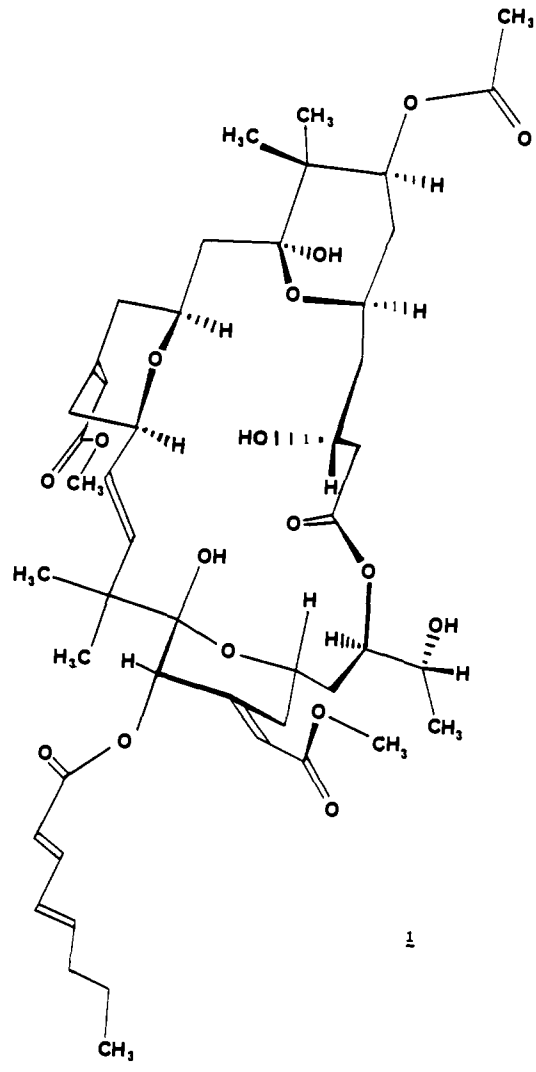
Marine animals of the phylum Ectoprocta (usually termed Bryozoa or Polyzoa) are colonial filter-feeders and each member (polypide) is enclosed in a separate unit (zooecium). Because of their superficial appearance Bryozoa are commonly known as sea-mats and false corals.[2] The genus *Bugula*[3] contains very prominent mosslike colonies, and *Bugula neritina* (Linnaeus) is well-known for its ability to attach to ship hulls.[4] Our initial report[5] that certain Bryozoa such as *B. neritina* L. contain anticancer constitutents, preliminary study of an adrenochrome-like pigment in the same species,[6] and isolation of indoles such as flustramines A and B from *Flustra foliacea*[7] appear to represent the only prior chemical investigations of Bryozoan metabolites.

We now report the structure of a remarkable[8] anticancer constituent of *Bugula neritina* designated bryostatin 1. The study began in 1968 with a Gulf of Mexico collection and has recently culminated in the structural elucidation of bryostatin 1 (**1**) by crystallographic and spectroscopic techniques. The biological activity of bryostatin 1 (**1**) is noteworthy. In the murine P388 lymphocytic leukemia (PS system)[9] macrocyclic lactone **1** shows 52–96% life extension at 10–70 μg/(kg/injection dose) levels and an ED_{50} of 0.89 μg/mL against the P388 in vitro cell line.

An initial methylene chloride extract prepared from 500 kg of wet animals was further fractioned by the solvent partition sequence 9:1 → 4:1 methanol–water with ligroin → carbon tetrachloride.[10] The carbon tetrachloride fraction (214 g) was purified by column chromatography using both Sephadex LH-20 and silica gel monitored by bioassay (PS system). Recrystallization from methylene chloride–methanol gave crystals of bryostatin 1 (**1**): melting at 230–235 °C. TLC (silica gel) R_f 0.7 (9:1 CH_2Cl_2–CH_3OH); EI MS *m/z* 886 ($M-H_2O$, $C_{47}H_{66}O_{16}$), exact mass 886.4376 amu (calcd. 886.4351 for $C_{47}H_{66}O_{16}$); FAB MS *m/z* 904 (M); $[\alpha]^{25}_D$ + 34.1° (*c* = 0.044, CH_3OH); UV (CH_3OH) λ_{max} 233 nm (ε 25 700) and 263 (ε 28 700); IR (KBr) 3470, 3400, 2970, 2950, 1735, 1716, 1700, 1640, 1600, 1433, 1385, 1365, 1245, 1160, 1100, 1080, 1000 cm^{-1}. Detailed high-resolution (400 MHz) NMR data has been included in a subsequent report.[11]



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Stout parallelepiped crystals were obtained from slow mixing of a layered solution of bryostatin 1 in methylene chloride under methanol. When maintained in the mother liquor, these crystals were found to belong to space group $P2_12_12_1$ with *a* = 21.782 (5), *b* = 20.428 (4) and *c* = 23.664 (6) Å and *Z* = 8. As the crystals dried, the *c* axis appeared to halve, and the relatively poor diffraction pattern conformed to $P2_12_12$.[12] A total of 5464 reflections was collected at −100 °C by using 1° ω scans and graphite-monochromated Mo Kα (0.71069 Å) radiation. Of these data, 3553 (65%) were judged observed ($|F_o| > 3\sigma(F_o)$) and used in subsequent calculations. By means of the program system

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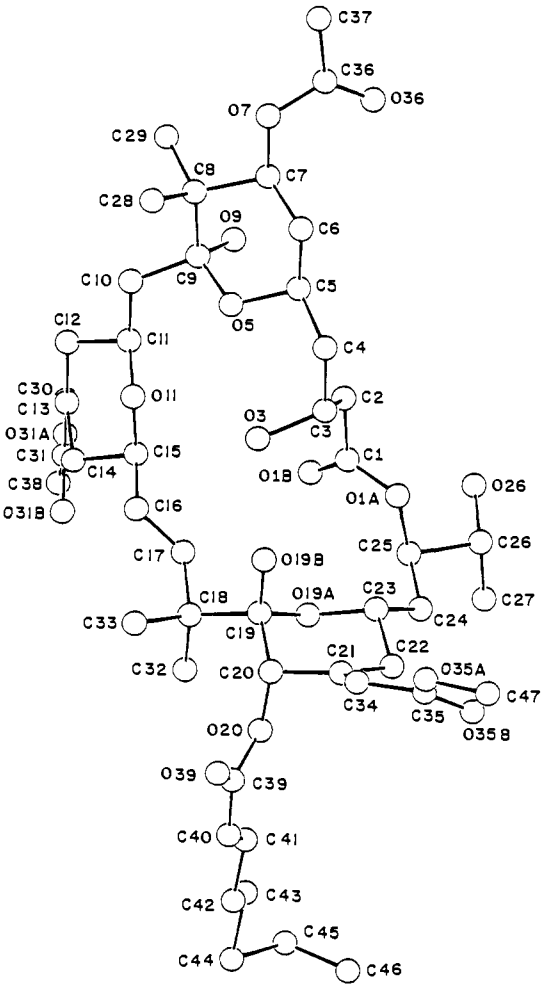

Figure 1. Computer-generated perspective drawing of the final X-ray model of bryostatin 1 less hydrogens.[17]

MULTAN 78[13] approximately 2000 phase sets were generated for the largest 350 normalized structure factors, and the sets were ranked with placement of equal weight on ABSFOM, PSIZERO, RESID, and NQEST. E-syntheses using one of the most promising sets provided two chemically sensible and identical 24-atom fragments that were related by a translation of approximately $c/2$. Efforts to extend this model by tangent formula recycling[14] and/or attempts to refine this model in least-squares analysis were unsuccessful. Thus, we returned to tangent formula recycling using a scale factor that was the average of the Wilson plot and the least-squares scale factors. An E-synthesis from this approach was strinkingly improved and showed 100 chemically sensible atoms in two essentially identical fragments. Further tangent formula recycling showed virtually all of the non-hydrogen atoms in both molecules of bryostatin 1.

Refinement of the final model by block-diagonal least-squares analysis alternating with Fourier syntheses using $(2F_{obsd} - F_{calcd})$ as coefficients[15] led to the placement of 128 non-hydrogen atoms in the two independent molecules and determination of the structure of bryostatin 1 as illustrated by **1**. The current X-ray model includes 59 anisotropic non-hydrogen atoms per molecule, the end atoms on the ester side chains which are isotropic (B's in the range 5–20 Å^2, average B approximately 2.5 Å^2), several solvent methanol molecules, and 114 hydrogens at calculated positions. The standard crystallographic residual (R factor) for this model has converged to 0.07 for the observed data.

As anticipated from the pseudosymmetry of the crystal, both independent molecules of bryostatin 1 have identical stereostructures and essentially identical conformations.[16] Figure 1 shows a perspective view of the X-ray model (less hydrogens) of crystalline bryostatin 1. The relative[17] stereochemical designations of the eleven chiral centers in bryostatin 1 are 3(R),5(R),7(S),9(S),11(S),15(R),19(S),20(S),23(S),25(R),26(R). The crystal conformation of bryostatin 1 defines a roughly scoop-shaped molecule with length 13 Å, width 8 Å, and height approximately 6 Å. Examination of molecular models suggests that there are other plausible conformations.

Bryostatin 1 may be considered as a 26-membered macrolide ring. Embedded in the 26-membered ring is a 20-membered cycle defined by taking the shorter path through the pyran oxygens rather than along the carbon chain. The longest chain of carbon atoms is 27 and has been used in the proposed numbering system. The oxygen substitution pattern, augmented by the *gem*-dimethyl substituents at carbons 8 and 18, suggests a polyketide biosynthesis for bryostatin 1. All three pyran rings are approximately in the chair conformation and each has a 4-position substituent that projects outward. All of the macrocycle substituents are equatorial with reference to the pyran rings. An intramolecular hydrogen bond appears between O19H and O3 (2.71, 2.71 Å), and two possible hydrogen bonds are found between O3H and O5 (2.84, 2.87 Å), and between O3H and O11 (3.00, 3.02 Å).

In the crystalline conformation oxygens O1, O3, O5, O11, O19A, and O19B are all on the interior of the large, oxygen-rich cavity in bryostatin 1. The size and shape of this cavity and the arrangement of oxygen atoms suggest that the molecule may have cation-binding capabilities, similar to the polyether antibiotics. The axial (*E*,*E*)-octa-2,4-dienoic acid substituent at C20 would be expected to enhance lipid solubility. An intriguing possibility is that this substituent could swing over the internal cavity by rotation about the C20–O20 bond and "seal" one side. The stereochemistry of the two acetylidene units in bryostatin 1 at C13 and C21 is such that the carbonyl oxygen points in the direction of increasing carbon number along the macrocycle. While these units are disubstituted at the β carbon, it is conceivable that they could function as Michael acceptors for biopolymer amine and/or thiol groups.

The extremely low dose level for antineoplastic activity suggests that this most interesting substance may possess other potentially useful pharmacological (and/or microbiological) activities and may become a valuable biochemical probe. Recent experiments with *Bugula neritina* L. fractions indicate that this marine animal[18] will yield additional[19] biosynthetic products with an exceptionally

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(17) The enantiomer of bryostatin 1 shown in Figure 1 was selected as follows: The method of Engel (Engel, D. W. *Acta Crystallogr., Sect. B* **1972**, *B28*, 1498–1509) was used to measure anomalous scattering effects due to oxygen and carbon ($\Delta f'' = 0.032$ and $\Delta f'' = 0.009$ electrons, respectively, for Cu Kα radiation). Seven groups of Bijvoet pairs that were expected to have the largest Bijvoet ratios were measured (three times) very slowly by using Cu Kα radiation and the same crystal that had been used for the Mo Kα data collection. Neighboring pairs, which in each case were centrosymmetric reflections, were measured in a manner similar to provide an empirical correction for absorption and other anisotropic effects. The absorption-corrected Bijvoet ratios that were obtained for each of the seven groups indicated the enantiomer shown in Figure 1. Efforts to determine the absolute configuration of bryostatin 1 are continuing.

(18) Alternatively, bryostatin 1 may have a dinoflagellate or other marine plant origin.

(19) To simplify the systematic description of bryostatin 1 and related natural products, we propose designating the fundamental ring system bryopyran. A more systematic description will appear in a subsequent communication.[11]

high level of antineoplastic activity (PS system). Studies concerned with uncovering such constituents and their mode of action will be continued. Presently, bryostatin 1 is being evaluated by using a selection of the U.S. National Cancer Institute's experimental solid tumor systems.

Acknowledgment. We are pleased to acknowledge very necessary financial support provided by Contract N01-CM-97262 with the Division of Cancer Treatment, NCI, National Institutes of Health, DHW, Grant No. CA16049-01 through 07 awarded by the National Cancer Institute, DHW, Mary Dell Pritzlaff, the 01in Foundation (Spencer T. and Ann W.), the Fannie E. Rippel Foundation, Eleanor W. Libby, the David Ware Waddell Foundation, Pearl Spear, and Robert B. Dalton. For other very helpful assistance we are pleased to thank Drs. J. D. Douros, J. J. Einck, D. Gust, J. L. Hartwell, R. R. Inners, L. W. Knapp, P. Lohavanijaya, M. I. Suffness, J. M. Schmidt, and J. Wtischel, Jr., M. A. Carlson, B. L. Norfleet, K. M. Welch, the Smithsonian Institution Oceanographic Sorting Center, and the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723). The Cornell chemists gratefully acknowledge support provided by NIH CA24487 (J.C.), and the National Science Foundation (predoctoral fellowship to E.A. and funds for the Cornell Chemistry Computing Facility).

Registry No. 1, 83314-01-6.

Supplementary Material Available: List of observed and calculated structure factor magnitudes and calculated phase angles (38 pages). Ordering information is given on any current masthead page. For other supplementary information please refer to ref 11 and 16.

1,3-Dilithiopropanes

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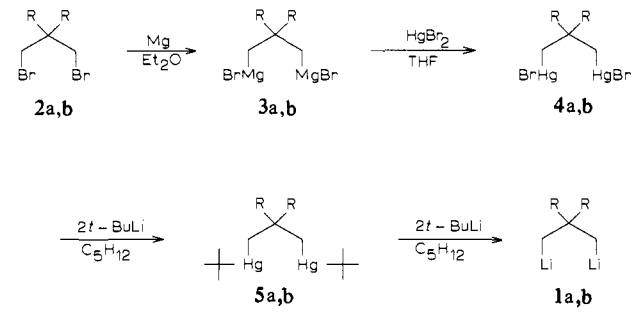
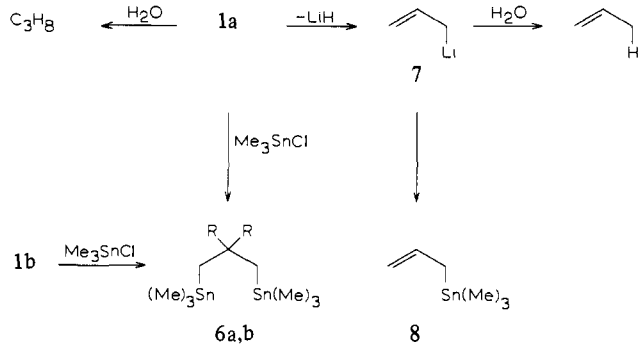
In a classical investigation, West and Rochow[1] established that dilithio derivatives can be prepared from α,ω-dibromoalkanes and lithium metal in ether only if four or more methylene groups separate the two functions. In the reaction of lithium with dibromomethane, only 6% bis(trimethylsilyl)methane was obtained after quenching with trimethylchlorosilane. On the other hand, 1,2-dibromoethane and 1,3-dibromopropane in a similar reaction gave no products indicative of the corresponding dilithio derivatives, although reaction did take place. More recently, Shimp and Lagow came to similar conclusions when performing this reaction with lithium vapor.[2] Likewise, our own attempts to prepare 1,3-dilithiopropane (**1a**, Scheme I) from 1,3-dichloropropane or 1,3-dibromopropane (**2a**) by reaction with lithium metal or *tert*-butyllithium were unsuccessful. Only aromatic,[3] conjugated,[4] and specially functionalized[5] 1,3-dilithium compounds have been described so far. We report here the first synthesis and some properties of the simple aliphatic 1,3-dilithium compound (**1a**) and its 2,2-dimethyl derivative **1b**.

Scheme I[a]



[a] a, R = H; b, R = Me.

Scheme II[a]



[a] a, R = H; b, R = Me.

A new approach to **1** was made possible by our finding[6] that 1,3-bis(bromomagnesio)propane (**3a**)[7] could, under special conditions, be prepared directly and conveniently from **2a**; by reaction of $HgBr_2$ in THF with **3a**, **4a** was obtained.[6] Addition of 2 equiv of *tert*-butyllithium to **4a** in pentane at 0 °C gave **5a** in a rapid reaction, after which precipitated LiBr was removed by filtration. The filtrate was a solution of pure **5a**[8] in pentane, but on attempts to isolate **5a**, it disproportionated to di-*tert*-butylmercury and a nearly insoluble (oligomeric?) material, presumably $(CH_2CH_2CH_2Hg)_n$. Addition of 2 further equiv of *tert*-butyllithium to the solution of **5a** lead to a slow reaction (several hours at room temperature) and the precipitation of a white powder that contained **1a**, together with **7** and LiH. **1a** was characterized by hydrolysis to propane and reaction with trimethylchlorostannane to **6a**;[6] in these reactions, **7** was converted to propene and **8**, respectively (Scheme II). Presumably, the preparation of **1a** can also be achieved by treating **4a** directly with 4 equiv of *tert*-butyllithium; so far, we did not explore this variant because it would furnish a precipitate that, besides **1a**, **7**, and LiH, also contains large amounts of LiBr.

We had anticipated that **1a** would be unstable and decompose spontaneously to allyllithium (**7**) by elimination of LiH. The analogous slow elimination of HMgBr had been observed in **3a** at room temperature;[6] a process that is facilitated through activation of the β hydrogen by the *two* carbon–metal bonds.[9] Normally, primary alkyllithium compounds eliminate LiH at a measurable rate only above ca. 100 °C.[10] However, the effectiveness of β-hydrogen activation in **1a** is quite spectacular: at 20 °C the half-life of **1a** is 1 h at the utmost, as estimated by monitoring the amount and ratio of propane and propene after

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