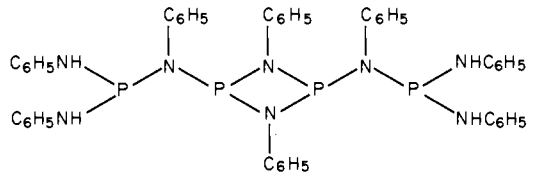
materials at temperatures at which the rate of trimer-dimer interconversion is appreciable. Experiments to find species which might catlyze the **1** ⇌ **2** interconversion reaction or to discover differently substituted aminophosphine systems in which the equilibrium is more facile are in progress currently.

Compounds **1** and **2**, through their relationship as shown in eq 4, represent two members of a novel oligomerization series involving species of the general formula $[(C_6H_5NH)PNC_6H_5]_n$. So far, no evidence for the monomer (n = 1) has been obtained. If the tendency toward formation of 1,3,2,4-diazaphosphetidine rings persists in the series, the series is limited and can exhibit besides the monomer (n = 1), dimer (n = 2), and trimer (n = 3), only tetramer (n = 4) $[(C_6H_5NH)PNC_6H_5]_4$ (**3**). Species of higher



3

n cannot exist unless structures which contain bond arrangements other than 1,3,2,4-diazadiphosphetidine rings occur. Intensive study of the conditions under which **3** might be formed and isolated seems warranted.

Acknowledgment. Support of this work in the form of sabbatical leaves for M.L.T. (Lake Forest College) and A.T. (Jundi Shapur University) and grants from the National Science Foundation (CHE 76-04290 and CHE 79-09497) and the University of Colorado Computer Center is gratefully acknowledged.

Supplementary Material Available: Tables of positional and thermal parameters for nongroup atoms and rigid group atoms (3 pages). Ordering information is given on any current masthead page.

Sceptrin, an Antimicrobial Agent from the Sponge *Agelas sceptrum*

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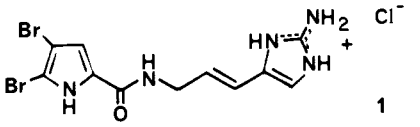
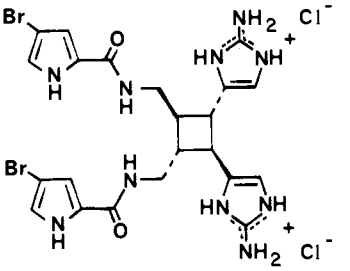
During a study of Caribbean sponges, we have examined several sponges of the genus *Agelas*, all of which gave ethanolic extracts having antimicrobial activity, in agreement with previous reports.[1] Prior studies by Minale et al.[2] resulted in the identification of 4,5-dibromo-2-cyanopyrrole as the antimicrobial constituent of the Mediterranean sponge *Agelas oroides*. *A. oroides* also contained 4,5-dibromopyrrole-2-carboxylic acid,[3] the corresponding amide, and oroidin (**1**).[4] In this communication, we report the structural elucidation of sceptrin (**2**), the major antimicrobial constituent of *Agelas sceptrum* (Lamarck).



1



2

Antimicrobial assays of the crude extracts of six *Agelas* samples revealed the presence of active compounds in all samples. When the crude extracts were partitioned between ethyl acetate and water, *A. sceptrum* was distinguished by the strong antimicrobial activity of the aqueous phase. *Agelas sceptrum*, collected at Glover Reef, Belize, was maintained frozen until required. The lyophilized sponge was extracted sequentially with hexane, dichloromethane, and methanol. The acetone-insoluble portion of the methanolic extract was twice chromatographed on Sephadex LH-20 by using first methanol and then 1:1 methanol/chloroform as eluants to obtain a fraction containing the antimicrobial material. This fraction was chromatographed on a LiChrosorb DIOL column by using 1:1 methanol/chloroform as eluant to obtain oroidin (**1**, 0.5% dry weight) and sceptrin (**2**, 2.1% dry weight). Traces of a colored impurity were removed by passing an aqueous solution of sceptrin through Sephadex G-10, after which sceptrin (**2**) (as the dihydrochloride) was crystallized from water. Sceptrin (**2**), mp 215-225 °C dec, $[\alpha]_D$ -7.4° (c 1.2, MeOH), had the molecular formula $C_{22}H_{24}Br_2N_{10}O_2 \cdot 2HCl \cdot nH_2O$.[5] The electron impact mass spectrum did not show a molecular ion, but the field desorption mass spectrum contained a triplet at m/z 619, 621, 623 $(C_{22}H_{25}Br_2N_{10}O_2)^+ \cdot$. The following spectral data indicated that sceptrin (**2**) was a symmetrical dimer of the 2-debromo derivative of oroidin (**1**): IR (KBr) 3350, 1680, 1625 cm^{-1}; UV (MeOH) 265 nm (ϵ 20850); ^1H NMR (Me_2SO-d_6) δ 2.29 (br s, 1 H), 3.10 (d, 1 H, J = 8 Hz), 3.42 (br s, 2 H), 6.66 (s, 1H), 6.97 (s, 1 H), 6.99 (s, 1 H), 7.33 (br s, 2 H), 8.59 (br t, 1 H, $J \simeq$ 5 Hz); ^{13}C NMR (D_2O) δ 160.8 (s), 145.7 (s), 123.9 (s), 121.6 (d), 111.6 (d), 108.3 (d), 95.2 (s), 41.6, 40.9, 36.9.

Sceptrin (**2**) formed small crystals in the monoclinic class, and accurate cell constants determined by a least-squares fit of 15 high angle reflections were a = 19.788 (8) Å, b = 13.337 (4) Å, c = 13.725 (7) Å, and β = 122.69 (2)°. Systematic extinctions ($h + k = 2n$), a calculated density of 1.63 g/cm^3, and the presence of chirality were uniquely accomodated by the space group $C2$, with four molecules of $C_{22}H_{26}Br_2Cl_2N_{10}O_2 \cdot 3H_2O$ per unit cell. All unique diffraction maxima with $2\theta \leq 100°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Cu Kα (1.54178 Å) radiation and a variable speed ω-scan technique. Of the 2172 unique reflections surveyed in this fashion, 1697 (78%) were judged observed [$F_o \geq 3\sigma(F_o)$] after correction for Lorentz, polarization, and background effects.

A phasing model was achieved by standard heavy-atom procedures.[6] The deconvolution of the Patterson synthesis gave the

(1) Burkholder, P. R. In "Biology and Geology of Coral Reefs, Biology I"; Jones, O. A., Endean, R., Eds.; Academic Press: New York, 1973; p 144 and references cited therein.

(2) Minale, L.; Cimino, G.; de Stefano, S.; Sodano, G. *Prog. Chem. Nat. Prod.* **1976**, *33*, 1.

(3) Forenza, S. L.; Minale, L.; Riccio, R.; Fattorusso, E. *Chem. Commun.* **1971**, 1129.

(4) After some confusion, the structure of oroidin (**1**) was accepted to be that shown.[2] An interest in solid-state photodimerization reactions prompted us to carry out a single-crystal X-ray diffraction analysis of oroidin. This study reconfirmed the structure shown and details can be found in the supplementary material.

(5) The elemental analysis of a sample dried at 110 °C over P_2O_5 required one molecule of water per sceptrin molecule while the X-ray study indicated three water molecules per sceptrin.

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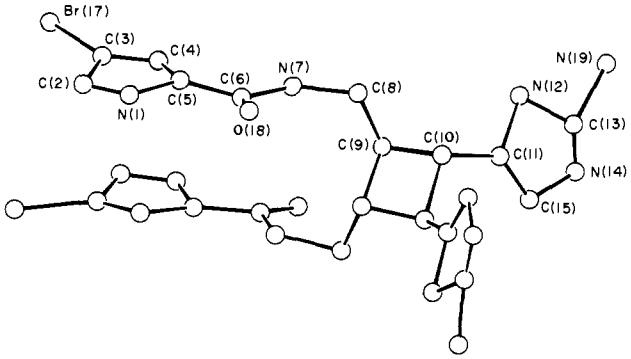

Figure 1. A computer generated perspective drawing of the final X-ray model of sceptrin (**2**). Hydrogens are omitted for clarity. Only one-half of the molecule is crystallographically independent; the other half is generated by the crystallographic twofold axis that bisects the cyclobutane ring.

Br positions. The remaining nonhydrogen atoms were located in subsequent electron density maps. Atom types were initially assigned by analysis of the thermal parameters and geometry. These identities were allowed to vary in alternate refinements with poorer agreement. Some of the hydrogens, including single hydrogens at N(12), N(14), and N(19), were located on a difference map, and the remainder were included at calculated positions. In general the hydrogen positions were not well defined, as was demonstrated by the insensitivity of the final residual to their inclusion. Full-matrix least-squares refinement with anisotropic temperature factors for the nonhydrogen atoms, isotropic hydrogens, and anomalous dispersion corrections have converged to a standard crystallogrphic residual of 0.090 for the structure and 0.094 for the enantiomer. The bromopyrrole fragments of both independent sceptrins show highly anisotropic thermal parameters with large atomic excursions perpendicular to the plane of the ring. All of the highest peaks in the final difference synthesis occur around the bromines. There may be some disorder in the crystal which is imperfectly described in our current model.

The twofold axis of the sceptrin molecule is coincident with the crystallographic twofold axis. Thus only half of the atoms in one molecule are independent, and the asymmetric unit of the cell contains two such independent $C_{11}H_{13}BrClN_5O$ groups. The configuration of the two independent molecules is the same. Their geometries and conformations are also similar but the esd's are large. N(1), N(12), and N(19) are all involved in apparent hydrogen bonds with the waters of crystallization, further confirming the identity of atom types. A drawing of the final X-ray model for one molecule of sceptrin (**2**) is given in Figure 1. Bond distance and angles agree well with generally accepted values.

Sceptrin (**2**) is related to debromooroidin by a head-to-head $[{}_{\pi}2_s + {}_{\pi}2_s]$ cycloaddition reaction. Since this would be an allowed photochemical reaction, we have tried a number of solid-state and solution photodimerizations of oroidin (**1**), without success. The biosynthesis of sceptrin (**2**) cannot be regarded as a simple photodimerization of debromooroidin for two reasons: there is insufficient light at the depth where *Agelas sceptrum* was found (from −20 to −30 m) and, more importantly, sceptrin is optically active while debromooroidin must be achiral.

Sceptrin (**2**) exhibited antimicrobial activity against *Staphylococcus aureus* (MIC 15 μg/mL), *Bacillus subtilis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Alternaria* sp. (fungus), and *Cladosporium cucumerinum*. The antimicrobial activity of sceptrin was considerably greater than that recorded for oroidin.

Acknowledgment. Field desorption mass spectral data were supplied by Professor Burlingame, U.C., Berkeley. The sponge material was identified by Dr. K. Rützler, Smithsonian Institution. The sponge was collected during a cruise on R/V Alpha Helix, supported by the National Science Foundation. This research was supported by grants from the National Institutes of Health (CA-24487 to J.C. and AI-11969 to D.J.F.) and a N.I.H. training grant (to D.V.E.).

Supplementary Material Available: Additional X-ray crystallographic data for sceptrin (**2**) and oroidin (**1**) (25 pages). Ordering information is given on any current masthead page.

(6) The following library of crystallographic programs was used: Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr.* **1970**, *B24*, 274 (MULTAN). Hubbard, C. R.; Quicksall, C. O.; Jacobson, R. A. "The Fast Fourier Algorithm and the programs ALFF, ALFFDP, ALFFT and FRIEDEL", USAEC Report IS-2625; Institute for Atomic Research, Iowa State University: Ames, Iowa, 1971. Busing, W. R.; Martin, K. O.; Levy, H. A. "A Fortran Crystallographic Least Squares Program", USAEC Report ORNL-TM-305; Oak Ridge National Laboratory: Oak Ridge, TN, 1965. Johnson, C. "ORTEP: A Fortran ThermalEllipsoid Plot Program", USAEC Report ORNL-3794; Oak Ridge National Laboratory: Oak Ridge, TN, 1965.

Isolation and Structure of Brevetoxin B from the "Red Tide" Dinoflagellate *Ptychodiscus brevis* (*Gymnodinium breve*)

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A dense growth, or bloom, of dinoflagellates can occur under certain favorable conditions, causing a phenomenon descriptively known as "red tide". Blooms of the dino flagellate *Ptychodiscus brevis* Davis (*Gymnodinium breve* Davis) have caused massive fish kills, mollusk poisoning, and human food poisoning along the Florida coast and in the Gulf of Mexico.[1] Numerous attempts have been made since 1968 to isolate the toxins from cultured cells;[2] however, discrepancies exist in the reported physical properties,[1,3-5] the main reason presumably being the difficulty associated with the separation and purification of the toxin mixture.

All of the dinoflagellate toxins characterized to date belong to the saxitoxin (STX)/gonyautoxin (GTX) group which are tricyclic compounds containing two guanidinium moieties.[1] In this communication we wish to report the structure of brevetoxin B (BTX-B) (**1**), the first member of an entirely new group of natural products, the "brevetoxins" (BTX).

Unialgal cultures of *P. brevis*, isolated during an outbreak at Florida in 1953, were grown in an artificial sea-water medium as described previously.[6] The cultures were incubated at 25 °C for 21 days under constant illumination with standard fluorescent

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(2) Spikes, J. J.; Ray, S. M.; Aldrich, D. V.; Nash, J. B. *Toxicon* **1968**, *5*, 171.
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(6) Gates, E. J.; Wilson, W. B. *Limnol. Oceanogr.* **1960**, *5*, 171-174.

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