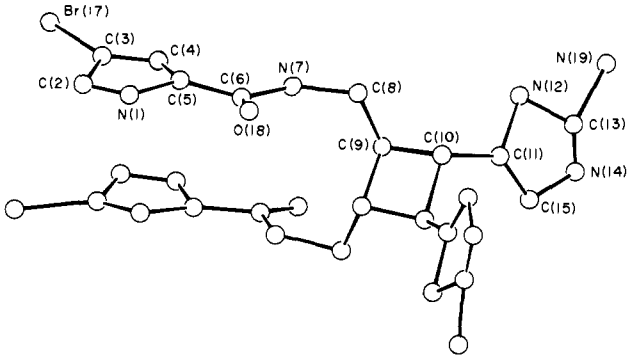

Figure 1. A computer generated perspective drawing of the final X-ray model of sceptrin (**2**). Hydrogens are omitted for clarity. Only one-half of the molecule is crystallographically independent; the other half is generated by the crystallographic twofold axis that bisects the cyclobutane ring.

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.

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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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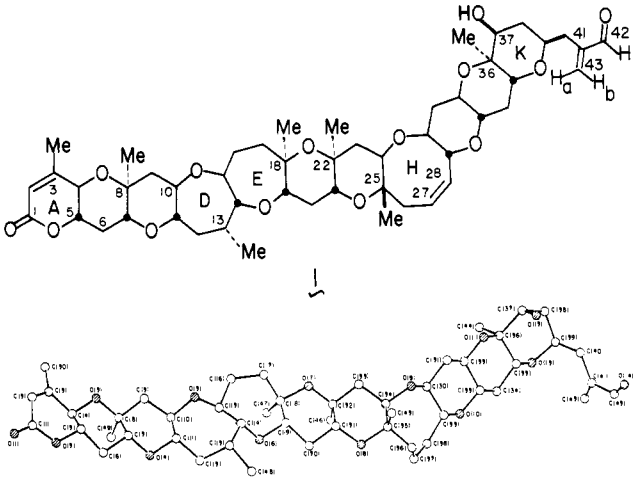


Figure 1. Computer generated perspective drawing of brevetoxin B (**1**). Hydrogens are omitted for clarity.

light. The medium (ca. 50 L contianing 5×10^8 cells) was acidified to pH 5.5 and extracted with diethyl ether to give 90 mg of crude brevetoxins. Repeated flash chromatography[7] of the crude toxic mixture with 5% methanol in diisopropyl ether (v/v) gave the following congeners: 0.8 mg of BTX-A, 5.0 mg BTX-B (**1**), and 0.4 mg of BTX-C.[8,9] Purity of the various toxins was checked by high-performance LC, Whatman Partisil ODS-2 analytical column, MeOH–H_2O (4:1). The fractions were monitored for ichthyotoxicity with the fresh water "zebra" fish *Brachydanio rerio*.[4,10]

Brevetoxin B (**1**) crystallized from acetonitrile as stout acicula. Preliminary X-ray photographs showed monoclinic symmetry, and precise lattice constants, calculated from a least-squares fit of 15 diffractometer measured 2θ vaues, were a = 12.510 (3) Å, b = 14.262 (2) Å, c = 13.746 (2) Å, and β = 106.21 (1)°. Systematic extinctions and the optical activity uniquely determined the space group as $P2_1$, and the density of 1.26 g/cc indicated one molecule of composition $C_{50}H_{70}O_{14}$ (M_r–894) formed the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer controlled four-circle diffractometer by using graphite monochromated Cu Kα (1.54178 Å) radiation. A total of 3331 reflections were collected, and after correction for Lorentz, polarization, and background effects, 3272 (98%) were judged observed [$|F_o| \geq 3\sigma(F_o)$].

A phasing model was arrived at by direct methods. Trial sets of phases were generated from a multisolution tangent formula approach,[11] and these were examined by the negative quartets procedure.[12] An E synthesis was calculated for the best set from this procedure, and a plausible 32-atom fragment was found. Successive cycles of tangent formula recycling[13] led to a 59-atom

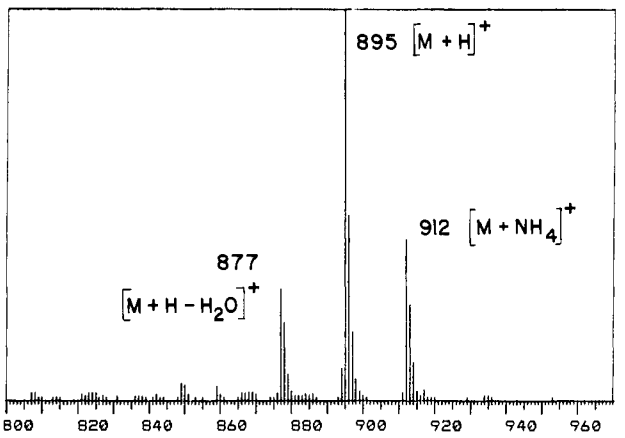
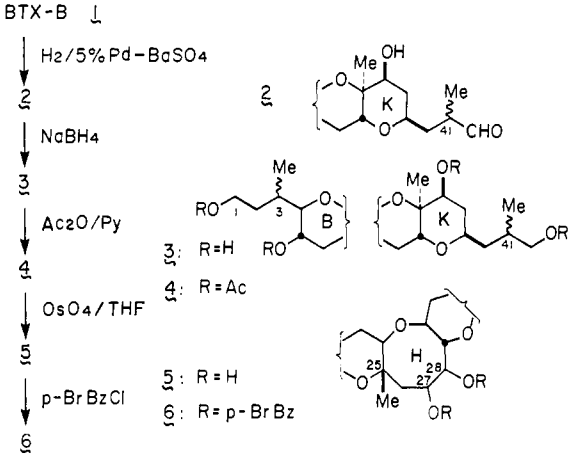


Figure 2. Desorption/chemical ionization mass spectrum (D/CI/MS) of brevetoxin B (**1**).[14,15]

Scheme I



fragment, and an electron density synthesis revealed all 64 nonhydrogen atoms. Block diagonal least-squares refinement followed by a difference electron density synthesis showed the majority of the hydrogen atoms. Block diagonal least-squares refinement with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a current residual of 0.062 for the observed reflections. Additional crystallographic details can be found in the supplementary materials.

Figure 1 is a computer generated perspective drawing of the final X-ray model for brevetoxin B (**1**). The enantiomer shown was selected on the basis of CD experiments described below. Brevetoxin is a slightly bent array of rings approximately 30 Å long, 6 Å wide, and 6 Å high. There are 11 rings, each containing one oxygen. All of the ring fusions are trans. There is a double bond in the eight-membered ring with cis stereochemistry. The stereochemical descriptions are C(4)*R*, C(5)*S*, C(7)*R*, C(8)*S*, C(10)*R*, C(11)*S*, C(13)*R*, C(14)*R*, C(15)*S*, C(18)*R*, C(19)*S*, C(21)*R*, C(22)*S*, C(24)*R*, C(25)*S*, C(29)*R*, C(30)*S*, C(32)*R*, C(33)*S*, C(35)*R*, C(36)*S*, C(37)*S*, and C(39)*S*. Bond distances and angles agree well with generaly accepted values, and there are no H bonds in the unit cell.

The major toxin BTX-B (**1**) exhibits the following physical constants: needles, mp 270 °C dec; $C_{50}H_{70}O_{14}$ by D/CI–MS[14,15] (Figure 2), m/z (relative intensity) 895 (100%) $[M + H]^+$, 912 (41%) $[M + NH_4]^+$; UV (MeOH) 208 nm (ϵ 16 000, $\pi\pi^*$ enal);

(7) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2972.
(8) BTX-A and BTX-B correspond, respectively, to "T_{46}" and "T_{47}" which were previously isolated[4] by dry column chromatography and high-performance LC.
(9) The BTX-B purified by flash chromatography crystallized out of acetonitrile during a preliminary NMR study.
(10) The lethal doses (LC_{50}) to kill these 0.2–0.6-g fish in 1 h were as follows: BTX-A, 3 ng/mL; BTX-B, 16 ng/mL; and BTX-C, 30 ng/mL.
(11) All crystallographic calculations were done on a PRIME 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were REDUCE and UNIQUE. Data reduction programs: Leonowicz, M. E. Cornell University, 1978. BLS78A, anisotropic block-diagonal least-squares refinement: Hirotsu, K.; Arnold, E. Cornell University, 1980. ORTEP crystallographic illustration program: Johnson, C. K. Oak Ridge, ORNL-3794, MULTAN-78 (locally modified): Main, P. et al. "A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data", University of York, England. For literature description of MULTAN, see: Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. B*, **1970**, *B26*, 274–285. Woolfson, M. M. *Acta Crystallogr., Sect. A* **1977**, *A33*, 219–225.
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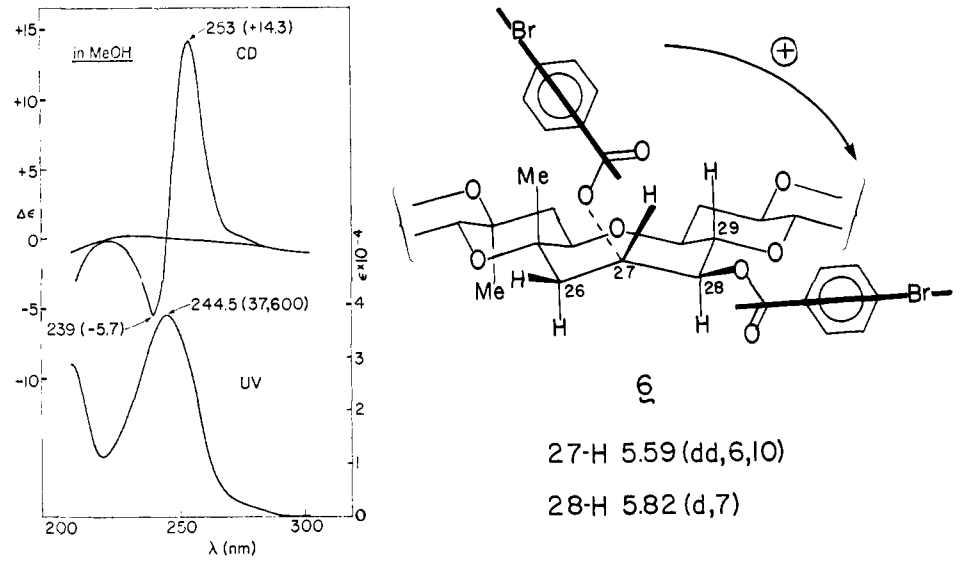

Figure 3. CD and UV spectra of **6**; positive exciton chirality of the 27,28-dibenzoate system in ring H.

CD[16] (MeOH) 225 nm ($\Delta\epsilon$ −3.93, ene–lactone $\pi\pi^*$), 257 (+6.77, ene–lactone $n\pi^*$), 329 (+0.16, enal $n\pi^*$); FTIR[17] (KBr pellet) 1737 cm^{-1} (lactone), 1691 (enal). NMR[18] data are as follows: 250-MHz ^1H NMR ($CDCl_3$) δ 1.04 (d, 3, J = 7 Hz, 13Me), 1.18 (s, 3, Me), 1.23 (s, 3, Me), 1.30 (s, 6, Me's), 1.31 (s, 3, Me), 1.97 (m, 3, 3-Me), 4.27 (ddq, 1, J = 10.6, 2.2 Hz, 4-H), 5.73 (dq. 1, J = 2.2, 1.0 Hz, 2-H), 5.78 (m, 2, 27-H and 28-H), 6.09 (d, 1, J = 1 Hz, 43-H_b), 6.32 (d, 1, J = 1 Hz, 43-H_a), and 9.53 (s, 1, 42-H); ^{13}C NMR ($CDCl_3$) δ 163.5 (C-1), 115.6 (C-2), 161.0 (C-3), 127.2 (C-27), 135.2 (C-28), 147.8 (C-41), 194.2 (C-42), and 135.7 (C-43); in addition 22 C–O signals were observed between δ 88.4 and 63.3, 12 CH_2's and 1 CH between δ 44.7 and 28.9, and 7 methyl signals appeared between δ 22.0 and 13.9.

It is to be noted that the UV and CD data are complementary, and hence both are necessary to reveal all of the transitions. The UV only shows a single short wavelength maximum corresponding to the enal, the other bands being too weak to be measured; in contrast, these weak UV transitions exhibit clear CD Cotton effects.

The absolute configuration of BTX-B (**1**) was determined by introducing a 1,2 dibenzoate system into the molecule via a 5-step sequence (Scheme I) and then applying the nonempirical dibenzoate chirality method:[19] (i) hydrogenation of 3.4 mg of BTX-B (**1**) with H_2/5% Pd–$BaSO_4$ in THF at 0 °C selectively reduced the 41-ene to give **2**; (ii) $NaBH_4$ reduction of the ene–lactone and aldehyde moieties gave the tetraol **3**; (iii) acetylation with Ac_2O/pyridine afforded the tetraacetate **4**; (iv) cis hydroxylation of the 27-ene located in the ring H was achieved with OsO_4 in THF.[20] The resultant diol **5** was derivatized with *p*-bromobenzyl chloride in pyridine to give 1.7 mg of the dibenzoate **6**. Each product was isolated and checked by ^1H NMR spectroscopy and D/CI-MS; the overall yield of the five-step sequence was 31%.

The absence of coupling between C-27 and C-28 protons in **6** suggests that their dihedral angle is close to 90°, thus indicating that the eight-membered ring adopts the crown conformation (Figure 3). Computer analysis using the Allinger MM2 force-field program also shows that the crown conformer is favored over the half-chair conformer by several kilocalories.[21]

As shown in Figure 3, the dibenzoate derivative **6** showed a typical split CD, and this established the chirality of the 27-OBz/28-OBz as being positive.[19] The absolute configuration of BTX-B is as shown in structure **1**.

Brevetoxin B is made up of a single carbon chain locked into a rigid ladderlike structure consisting of 11 contiguous trans-fused ether rings. There is no precedent for this extraordinary structure, and in addition, a plausible biogenetic scheme is not obvious. Structural studies of the minor toxins, BTX-A and BTX-C, are in progress.

Acknowledgment. The work at Cornell University was supported by NIH Grant CA 24487 and a training grant to Donna Van Engen. Studies at Columbia University were supported by NIH grant AI 10187.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, bond angles, observed and calculated structure factors (25 pages). Ordering information is given on any current masthead page.

(16) Jasco J-40 CD.
(17) IBM FTIR/85.
(18) Bruker WM-250 NMR.
(19) (a) Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989–3991. (b) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257. (c) "Exiton Coupled Circular Dichroism–Application in Organic and Bioorganic Stereochemistry"; University Science Books: Mill Valley, Ca, in press.
(20) Molecular models show that OsO_4 can approach only from one side.
(21) Allinger's MM2 program was obtained from the Quantum Chemistry Exchange (QCPE Program No. 395); we are indebted to Professor W. C. Still for the computer calculations.

High-Energy Fragmentation of Chlorophyll *a* and Its Fully Deuterated Analogue by ^{252}Cf Plasma Desorption Mass Spectrometry

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This report discusses results of the interaction of fast heavy ions (nuclear fission fragments from ^{252}Cf decay) with thin films of chlorophyll *a* (Chl *a*). Previous studies on Chl *a* by ^{252}Cf-plasma desorption mass spectrometry (^{252}Cf PDMS)[1] were directed to

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