bromide with NBS and debromination with zinc–copper couple, potassium iodide, and iodine in DMF (24%).[6,16] Again, C_{2v} symmetry was evident from the NMR spectra.[17] The colorless crystalline substance (mp 27–29 °C) exhibits the following electronic spectrum: λ_{max} cyclohexane 218 (ϵ 8900) and 318 (3000). Photoelectron spectroscopic measurements on **1** will be reported elsewhere. Heating a $CDCl_3$ solution of **1** to 80 °C induces clean rearrangement to **16**[18] ($t_{1/2}$ ~7 h). The same isomerization can be achieved more rapidly by irradiation with a TLC UV lamp (λ 254 nm). Accordingly, **1** finds [1,3] sigmatropic migration to be most accessible from its ground and excited states.

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(17) ^1H NMR (300 MHz, $CDCl_3$) δ 6.03–5.92 (m, 4 H), 5.80–5.70 (m, 4 H), 2.60–2.50 (br d, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) ppm 134.4, 123.1, 32.9.

(18) Paquette, L. A.; Kukla, M. J.; Ley, S. V.; Traynor, S. G. *J. Am. Chem. Soc.* **1977**, *99*, 4756.

Structure of Brevetoxin A (GB-1 Toxin), the Most Potent Toxin in the Florida Red Tide Organism *Gymnodinium breve* (*Ptychodiscus brevis*)

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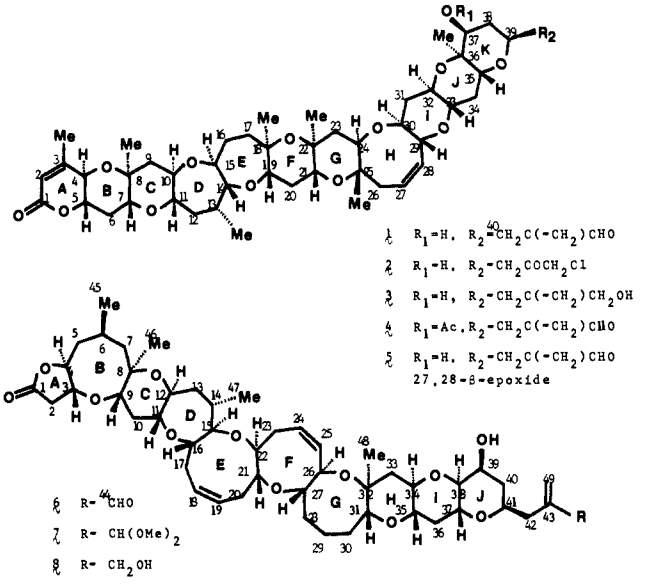
The dinoflagellate *Gymnodinium breve*[1] is the red tide causing organism responsible for massive fish kills and human intoxications including the so-called neurotoxic shellfish poisoning (NSP) in the Gulf of Mexico.[2] To date the structures of five polyether-type toxins, brevetoxin B (GB-2 toxin) (**1**),[3] brevetoxin C (**2**),[4] GB-3 toxin (**3**),[5] GB-5 toxin (**4**),[6] and GB-6 toxin (**5**),[6] have been established by X-ray crystallography and chemical and spectral correlations. The structure elucidation of the most potent ichthyotoxin, brevetoxin A[7] (LC_{100} 4 ng/mL to guppies), has preoccupied several groups, and a speculative structure was reported by a joint US–Japan group on the basis of NMR and mass spectral data.[8] The toxin is of particular interest not only because



it is the most potent toxin of this family, but also because it uniquely binds to sodium channels on excitable membranes.[9]

Brevetoxin A (**6**) was isolated from the cultured cells of *G. breve* by partition and successive chromatographic separations.[10] It forms fine prisms, mp 197–199 °C/218–220 °C (double melting point) from acetonitrile.[5] High-resolution FAB mass spectrometry gave the molecular formula $C_{49}H_{70}H_{13}$ (MH^+, m/z 867.4894; found, m/z 867.4927; $MH^+ - H_2O$, m/z 849.4789; found, m/z 849.4788). The ^1H and ^{13}C NMR spectra showed the presence of two secondary and two tertiary methyl groups, an α-methylene aldehyde, two disubstituted cis double bonds, and a carbonyl group. The IR absorption at $\nu(CH_2Cl_2)$ 1790 cm^{-1} suggested that the non-aldehydic carbonyl belongs to a γ-lactone. On the basis of extensive spin–spin decoupling, proton–proton coupling correlation (COSY), and proton–carbon correlation spectroscopy (hetero-COSY) experiments, we recently reported the partial structures shown in Figure 1 for **6**.[11] Due to the absence of certain signals in the COSY spectra and the discontinuity of proton couplings at the quarternary carbons, we were unable to connect these fragments with reasonable certainty. Although **6** was crystalline,[5] its X-ray analysis has not yet been successful. In an attempt to circumvent this difficulty, **6** was converted to a dimethyl acetal **7**, prisms, mp 233–235 °C, by treatment with methanol in the

(1) Some authors are using the newly proposed name *Ptychodiscus brevis* (Steidinger, K. A. "Toxic Dinoflagellate Blooms"; Taylor, D. L., Seliger, H. H., Eds.; Elsevier/North Holland: New York, 1979; pp 435–442), but a question has been raised regarding the appropriateness of the taxonomical change (e.g., Dodge, J. D. "Marine Dinoflagellates of the British Isles"; Her Majesty's Stationary Office: London, 1982; p 108). Present authors will use the traditional name until the matter is solved.

(2) Shimizu, Y. "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. I, pp 1–42. See also a collection of symposium papers on brevetoxins in: *Toxicon* **1985**, *23*, 4.

(3) Lin, Y. Y.; Risk, M.; Ray, S. M.; VanEngen, D.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775.

(4) Golik, J.; James, J. C.; Nakanishi, K. *Tetrahedron Lett.* **1982**, *23*, 2535–2538.

(5) Chou, H. N.; Shimizu, Y. *Tetrahedron Lett.* **1982**, *23*, 5521–5524.

(6) Chou, H. N.; Shimizu, Y.; Van Duyne, G.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 2865–2868.

(7) There is considerable confusion over the names of the toxins. GB-1 and GB-2 toxins were first reported in crude form by Alam et al. (Alam, M.; Trieff, N. M.; Ray, S. M.; Hudson, J. E. *J. Pharm. Sci.* **1975**, *64*, 865–867) and purified GB-2 with detailed spectroscopic data and structural information by Shimizu et al. (Shimizu, Y.; Alam, M., Fallon, W. F. "Proceedings of Food–Drugs from the Sea 1974"; Webber, H. H., Ruggieri, G. D., Eds.; Marine Technology Society: Washington, DC, 1976; pp 238–251 and ref 2). Later, supposedly identical compounds were reported under different names: T34, T47, and brevetoxin-B for GB-2 toxin and T46 and brevetoxin A for GB-1 toxin. Although we have been using the name GB-1 as the toxin preceding GB-2 toxin since 1974, here we decided to take an initiative to use brevetoxin A in order to avoid further confusion.

(8) Tempesta, M.; Golik, J.; James, J. C.; Nakanishi, K.; Pawlak, J.; Iwashita, T.; Gross, M. L.; Tomer, K. B. "The 1984 International Chemical Congress of Pacific Basin Societies"; Honolulu, HI, dec 16–21, 1984; Abstr. 10E 45. Nakanishi, K. *Toxicon* **1985**, *23*, 473–479. Proposed is a structure with the ring system 5(lactone)/6/6/7/11/8/8/6/6/6 and a trans double bond on the 11-membered ring. One of the major discrepancies is in the B-ring which was postulated as six membered with an angular methyl group.

(9) Catterall, W. A.; Risk, M. *Mol. Pharmacol.* **1981**, *19*, 345–348.

(10) The cells were extracted with methylene chloride, and the extract was partitioned between petroleum ether and 90% methanol. The methanolic extract was chromatographed on SiO_2 first with methylene chloride–benzene–methanol (40:5:1), and then with methylene chloride–ethyl acetate–methanol (5:3:0.1). After removal of brevetoxin B by crystallization, **6** was purified by HPLC [normal-phase SiO_2, isooctane–99% isopropyl alcohol (4:1)] in a yield of 1.2 mg from 10^9 cells. GB-7 (**8**) was separated from GB-3 toxin fraction[3] by HPLC [normal-phase SiO_2, isooctane–99% isopropyl alcohol (4:1)].

(11) Chou, H. N.; Shimizu, Y.; Van Duyne, G. D.; Clardy, J. "The Third International Conference on Toxic Dinoflagellate Blooms"; Anderson, D., Baden, D., Eds.; Elsevier: New York, 1985; in press.

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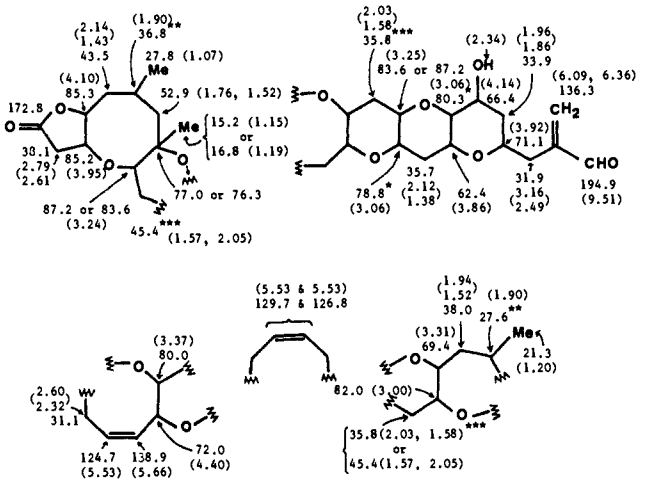

Figure 1. Partial structures of **6** proposed by COSY, heteroCOSY, and decoupling experiments. The attached numbers denote ^{13}C NMR (^1H NMR) chemical shifts, ppm in CD_2Cl_2.[11] The signals with asterisks are interchangeable.

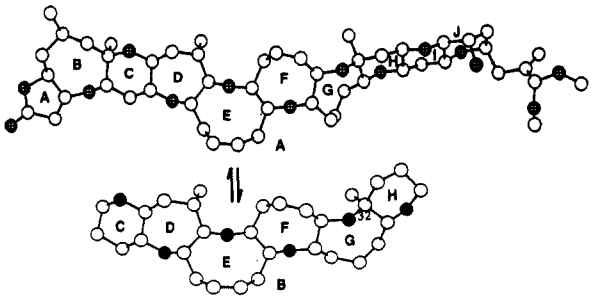

Figure 2. (A) Computer-generated perspective drawing of the final X-ray model of compound **7**. Hydrogens are omitted for clarity, and no absolute configuration is implied. Oxygen atoms are cross-hatched. (B) Partial perspective drawing of the molecule with ring G in crown form.

presence of Dowex 50W-X8 (H^+ form) at 45 °C for 1 h. Since **7** yielded **6** quantitatively upon treatment with aqueous acid, there was no structural alteration of the rest of the molecule during the acid treatment. the ^1H NMR spectrum of **7** is very similar to that of **6** except for the signals associated with the acetal moiety.

Compound **7** crystallized in the monoclinic space group $C2$ with $a = 27.249$ (5), $b = 10.572$ (2), $c = 21.739$ (4) Å, and $\beta = 128.85$ (2)°. One molecule of composition $C_{51}H_{76}O_{14}$ formed the symmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Cu Kᾱ radiation (1.541 78 Å) and variable speed, 1° ω-scans. Of the 3504 reflections measured in this fashion, 2777 (79%) were judged observed ($|F_o| \geq 3\sigma(F_o)$).[12] The structure solution was difficult, but eventually a phasing model was found. Full-matrix least-squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.050 for the observed reflections. Additional crystallographic details are available and are described in the supplementary material.

Figure 2 is a computer-generated perspective drawing of the final X-ray model.[13] This structure is in excellent agreement with the structural information obtained from NMR analysis. A remarkable feature of the structure is the unprecedented presence of all ring systems from five- to nine-membered in a single molecule. Another conspicuous structural feature is that in the crystal form, the molecule has a 90° twist at ring G. The molecule is essentially composed of two perpendicularly linked polycyclic sheets, rings A–F and rings H–J. In the solid state, ring G has the boat–chair (BC) form instead of the crown form. In brevetoxin B and its derivatives, the molecules are essentially planar. It is speculated that **6** undergoes a rather slow conformational change between the BC and crown form in solution.[14] This would explain the unusual peak broadening or disappearance of certain signals, particularly those of the protons and carbons around ring G, experienced in the NMR studies. In retrospect, this is the major cause of our failure to assign the structure for the central portion of the molecule in spite of extensive use of modern NMR techniques. Molecular mechanics calculations[15] indicate that the BC form is more stable than the crown form by a modest 2.9 kcal/mol. Most of the energy difference is associated with angle strain at the G-ring oxygen and C-32.

The structure elucidation of **6** also establishes another natural toxin GB-7 (**8**)[10] mp 295 °C dec, since **8** was determined to be the alcohol derivative of brevetoxin A on the basis of ^1H and ^{13}C NMR spectra. The spectra were superimposable except that the signals belonging the α-methylene aldehyde are replaced by those of α-methylene carbinol [δ (CD_2Cl_2) 4.05 (CH_2OH), 4.93 and 5.08 (terminal methylene)].

Acknowledgment. We are grateful to the National Institutes of Health Grants, GM 28754 (to Y.S.) and CA 24487 (to J.C.), New York State Sea Grant (to J.C.), and the support of the NSF Northeast Regional NMR Facility at Yale University funded by CDP-7916210. The FAB mass spectra were taken by Dr. Catherine E. Costello, at the High Resolution Mass Spectrometry Facility, Department of Chemistry, Massachusetts Institute of Technology, supported by the National Institutes of Health Grant RR 00317 (Principal Investigator: Professor K. Biemann), which is greatly appreciated.

Supplementary Material Available: Tables of ^{13}C and ^1H NMR signal assignment for **6** and fractional coordinates, thermal parameters, interatomic distances, interatomic angles, and torsional angles for **7** (10 pages). Ordering information is given on any current masthead page.

(12) The crystallographic programs employed are described in: Roll, D. M.; Chang, C. W. J.; Scheuer, P. J.; Gray, G. A.; Shoolery, J. N.; Matsumoto, G. K.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 2916–2920.

(13) The X-ray analysis did not afford the absolute configuration, which is currently under investigation.

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Pentacyclo[12.2.2.2^{2,5}.2^{6,9}.2^{10,13}]-1,5,9,13-tetracosatetraene and Its Reaction with AgOTf. Synthesis of a Square-Planar d^{10} Organometallic Complex

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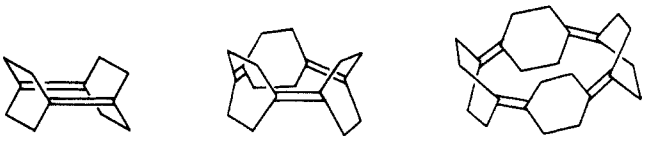
We reported recently on the synthesis of tetracyclo-[8.2.2.2^{2,5}.2^{6,9}]-1,5,9-octadecatriene (**2**) and remarked that this



1 **2** **3**

substance might be considered as a member of a class of compounds made of n six-membered rings joined by double bonds at their C1 and C4 positions to form a large overall ring.[1] The smallest member of the class, diene **1**, has also been reported recently.[2] We felt that the third member of the class, tetraene

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(2) (a) Wiberg, K. B.; Matturro, M.; Adams, R. *J. Am. Chem. Soc.* **1981**, *103*, 1600. (b) Wiberg, K. B.; Matturro, M. G.; Okarma, P. J.; Jason, M. E. *J. Am. Chem. Soc.* **1984**, *106*, 2194.

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