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ISOLATION AND STRUCTURES OF TWO NEW POLYCYCLIC ETHERS FROM GYMNODINIUM BREVE DAVIS (=PTYCHODISCUS BREVIS)

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Abstract: Two new polycyclic ethers were isolated from the unialgal culture of red tide dinoflagellate, Gymnodinium breve Davis (Syn. Ptychodiscus brevis) and their structures elucidated.

Deleterious red tide organism, Gymnodinium breve (=Ptychodiscus brevis) is the causative dinoflagellate responsible for massive fish kills and human intoxications along the Gulf Coast of Florida.[1] Already three toxins have been isolated from the organism and their conspicuous linear polycyclic ether structures as represented by brevetoxin-B (=GB-2, T17) 1 elucidated.[2-6] In this communication we wish to report two more new toxins, GB-5 2 and GB-6 3 from the cultured cells.

GB-5 toxin 2 is an amorphous minor component, which was first eluted slightly ahead of 1 in normal phase chromatography (SiO_2, benzene-ethyl acetate 1:3) overlapped with previously reported GB-1 toxin of still undetermined structure[5], and subsequently purified by SiO_2 preparative TLC in a solvent system, hexane-acetone 5:1. The pmr spectrum of 2 was almost superimposable with that of 1, except for considerable down-field shifts of the secondary alcohol methine proton (H-37, δ3.80 ppm → δ5.10 ppm, d,d) and the neighboring 36-methyl group (δ1.22 ppm → δ1.30 ppm), and the appearance of an acetyl methyl signal (δ2.12 ppm). The coupling patterns of H-37 and adjacent protons remain identical, therefore, we concluded the compound is simply 37-O -acetate of brevetoxin-B.

GB-6 toxin 3 is a crystalline compound, mp 295-297°C (sinters at 255°C), which was also isolated as a minor component by preparative TLC (SiO_2, benzene-ethyl acetate 1:1) after removal of co-existing brevetoxin-B by crystallization (Rf = 0.37 cf Rf = 0.42 for brevetoxin-B, benzene-ethyl acetate 1:3, HPKF plate, Whatman).

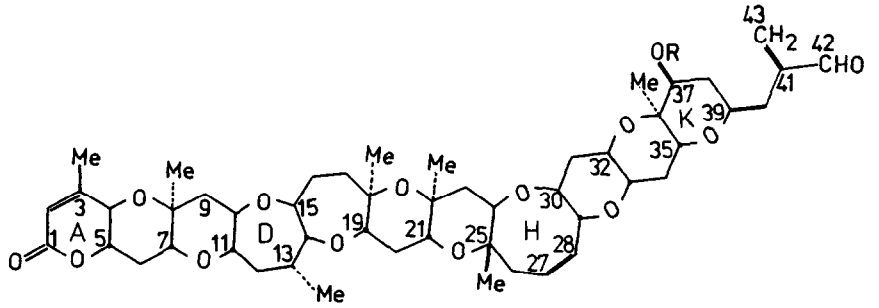
The high resolution pmr (500 MHz) and cmr (125 MHz) spectra of 3 showed signals for five tertiary, one secondary and one olefinic methyl groups, and α-methylene aldehyde, and other signals very close to those of 1. In the spectrum of 3, however, the signals of disubstituted olefinic protons, H-27 and H-28, are absent, and instead, a set of new signals are seen at δ2.87 and δ3.03 ppm. Chemical shift changes are also seen with the surrounding protons and carbons, particularly 25-methyl proton signal, which is shifted to down-field by 0.1 ppm. Final proof for the structure has been provided by X-ray crystallography.

Table 1. Comparison of significant carbon and proton chemical shifts of brevetoxin-B, 1 and GB-6, 3.

	1	3
Ring A C-1, -2	163.58, 115.90 (5.64)	163.79, 115.93 (5.64)
Ring H C-27, -28	127.39, 135.82 (5.72 5.72)	57.48, 51.89 (3.03 2.87)
α-Methylene C-43	136.09 (6.29 6.04)	136.16 (6.28 6.03)
Aldehyde C-42	194.64 (9.54)	194.74 (9.47)
3-Me	17.31 (1.91)	17.29 (1.91)
13-Me	18.62 (0.99)	18.62 (0.99)
Other Methyls	22.04, 20.22, 18.33, 16.02, 14.14 (1.25, 1.23, 1.23, 1.16, 1.12)	22.04, 20.48, 17.49, 14.11, 16.01 (1.29, 1.26, 1.24, 1.21, 1.17)

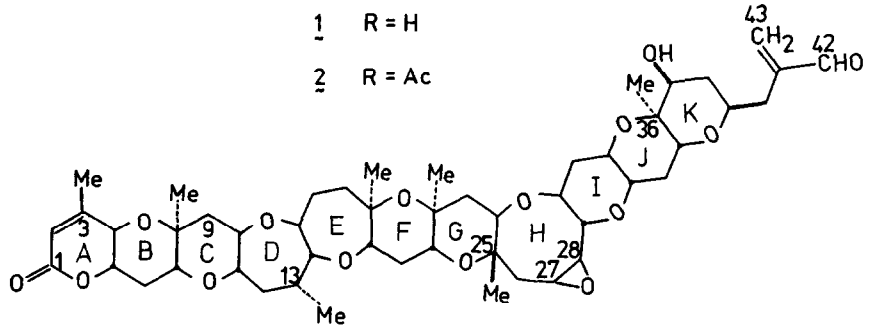
Compound 3 formed lovely crystals upon slow evaporation of a MeOH or acetonitrile solution. A crystal from MeOH was used in the single crystal X-ray diffraction analysis. Preliminary X-ray photographs showed monoclinic symmetry, and accurate lattice constants of a=12.552 (2), b=14.319(2), c=13.766(2) Å, and β=106.52(1)°. These cell constants were strikingly similar to those obtained for brevetoxin-B, 1-a=12.510(3), b=14.262(2), c=13.746(2) Å, and β=106.21(1)°.[4] Systematic extinction, crystal density and optical activity were uniquely accommodated in space group $P2_1$ with one molecule of composition $C_{50}H_{70}O_{15}$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 100^\circ$ were collected on a computer controlled four-circle diffractometer using variable speed, 1° ω-scans and graphite monochromated Cu Kα radiation (1.54178 Å). Of the 2567 reflections measured in this way, 1951 (76%) were judged observed ($F_o \geq 3\sigma(F_o)$) after correction for Lorentz, polarization and background effects.[7] The structure was solved making use of the fact that 1 and 3 were isostructural. Initial phases from the BTX-B structure were subjected to tangent formula refinement[8] with the 3 data. An E-syntheses produced in this fashion showed all of the nonhydrogens atoms of the central nine rings. The structure was completed with ΔF-syntheses. Block diagonal least-squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.087 (R_w=0.087) for the observed data.[9]

Figure 1 is a computer generated perspective drawing of the final X-ray model of 3. Hydrogens were omitted for clarity, and the absolute configuration was chosen to agree with 1.[4] The derived structure, 27S, 28R-epoxybrevetoxin-B may have some biosynthetic significance. Since the polycyclic ether structures are probably formed by the consecutive epoxide openings of a polyepoxidized polyene intermediate as seen in the biosynthesis of polyether antibiotics,[10] the epoxide on the eight-membered ring can be considered as a residual one which did not participate in the cyclization process.

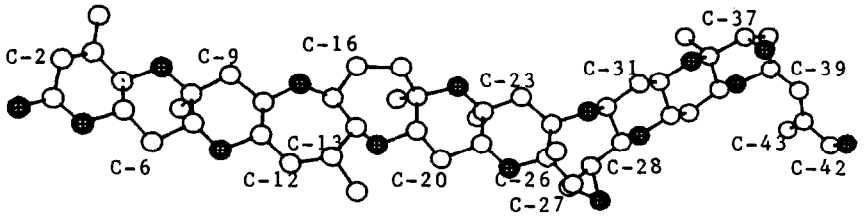


1 R = H

2 R = Ac



3



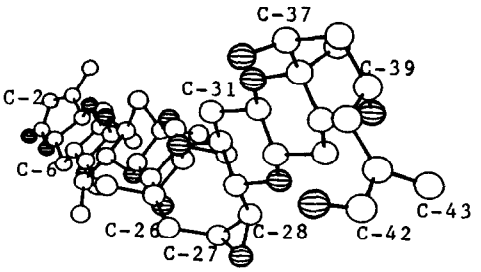


Fig. 1. Computer-generated perspective drawings of GB-6 toxin from two different angles.

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