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A NEW POLYETHER TOXIN FROM GYMNODINIUM BREVE DAVIS

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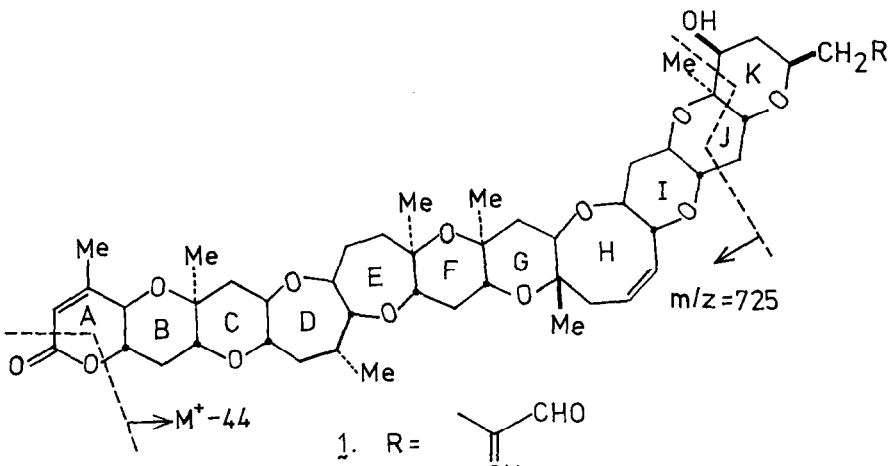
Summary: A new ichthyotoxic toxin was isolated from the unialgal culture of the deleterious Florida red tide organism, Gymnodinium breve Davis (Syn. Ptychodiscus brevis), and its structure was elucidated.

The toxic dinoflagellate, Gymnodinium breve (Syn. Ptychodiscus brevis) is the causative organism of red tides along the Gulf coast of Florida, which accompany massive fish kills and human intoxications. Since 1968, a number of pharmacological studies and attempted characterizations of the toxins from this unicellular alga have been reported.[1] Among the recent works, Alam, et al.[2] reported toxins T_1 and T_2 from the cultured cells. The toxin T_2 was the major toxin which was assigned a molecular formula of $C_{41}H_{59}NO_{10}$ and reported to have UV absorption maxima at 260, 267 and 270 nm. Later this toxin was further purified to a compound with a single UV absorption maximum at 213 nm.[3] The compound tentatively called GB-2 toxin (G. breve toxin-2), was assumed to be a polyalkyl ether with an aldehyde function from its spectroscopic data including 270 MHz ^{1}H-nmr spectrum.[1,3] Baden, et al.[4] isolated two toxins designated T_{17} and T_{34}. Risk, et al.[5] reported that they were unable to find the T_2 toxin fraction, instead they isolated T_4 toxin which was further separated into T_{46} and T_{47} toxins. Subsequently, Lin, et al.[6] succeeded in X-ray crystallography of T_{47} (now named brevetoxin-B) which revealed an unprecedented linear polyether structure, 1. Most recently, Golik, et al.[7] of the same group reported another toxin, brevetoxin-C, 2, with an α-chloroketone group instead of α-methylene aldehyde on the same polyether backbone.

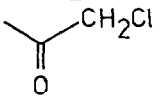
From the cultured cells[8] we have isolated three crystalline toxins. The major component was the previously reported GB-2 toxin, mp 295-297° (decomp.) and the others designated GB-1, mp 197-199°, and GB-3, 3. The approximate yields were 1.4 mg of GB-1, 9.8 mg of GB-2, and 3.0 mg of GB-3 from 1.5×10^9 cells after purification by medium pressure chromatography and preparative TLC. In spite of all the aforementioned confusion, GB-2 (=T_2) is now proved to be identical with T_{34}[7,9] and brevetoxin-B (=T_{47}) by comparison of the high resolution ^{1}H-nmr spectra,[10] and hereafter we call it brevetoxin-B (BTX-B).

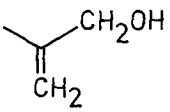
GB-3, 3, was obtained as needles from acetonitrile, mp 291-293°. It was very unstable in $CHCl_3$ solution and easily decomposed to several compounds.

This fact may explain the reason why some other groups did not isolate the compound and the confusion of the isolation of different toxic components. The EI mass spectrum of the compound at 16 ev showed the major fragment ions: m/z 852 (8%), 848 (4%), 725 (28%), 681 (72%), 349 (48%), 291 (32%) and 109 (100%). The highest mass m/z 852 is two mass units higher than the highest mass observed in the EI-MS of 1, m/z 850 [$C_{50}H_{70}O_{14}$, M^+-CO_2].[3] Furthermore, both spectra showed major fragment peaks at m/z 725 and 681, probably resulted from J/K ring fission (M^+-169) and a subsequent CO_2 loss (M^+-169-44) respectively, indicating both compounds have the same maximum absorption near the end absorption λmax (MeOH) = 204 nm (ε = 11,700) due to a single chromophore of conjugated lactone.



1. R =

2. R = 

3. R = 

The 500 MHz ^{1}H-nmr spectrum of 3 is virtually identical with that of 1 in the methyl signal region.[6] Thus there are seven methyl signals δ($CDCl_3$) ppm: 1.04 (d,3H), 1.18 (s,3H), 1.25 (s,3H), 1.29 (s,3H), 1.30 (s,3H), 1.31 (s,3H), and 1.97 (s,3H), which are clearly resolved in the DMSO-d_6 spectrum, δppm: 0.94 (d,3H), 1.08 (s,3H), 1.10 (s,3H), 1.19 (s,3H), 1.21 (s,3H), 1.24 (s,3H), and 1.88 (s,3H). The corresponding signals in the spectrum of 1 in DMSO-d_6 are δppm: 0.94 (d,3H), 1.08 (s,3H), 1.09 (s,3H), 1.19 (s,3H), 1.21 (s,3H), 1.24 (s,3H), and 1.88 (s,3H). However, the spectra differed in the lower field. The aldehyde signal (δ9.53) of 1 is conspicuously absent in the spectrum of 3, and

is replaced by a singlet signal of two protons (δ4.09, 2H) in the spectrum. Of five olefinic proton signals δ($CDCl_3$): 5.72 (m,1H), 5.78 (m,2H), 6.08 (d,1H), 6.31 (d,1H), the signals due to a terminal methylene structure shifted remarkably to the upper field (δ4.95 and 5.11) compared to those in the spectrum of 1 (δ6.08 and 6.31).

All these data point to the structure of dihydrobrevetoxin-B, in which the aldehyde group is replaced with a hydroxymethylene function. Confirmation of this structure was then achieved by the correlation of 3 and 1 (BTX-B). Compound 1 was thus reduced with $NaB(CN)H_3$ under mild conditions. The reduction product was identical with 3 in ^{1}H-nmr spectrum, HPLC and TLC.

In the ichthyotoxicity study, the new compound showed toxicity comparable with that of BTX-B (LC_{100} = 37 ng/ml for 3 and 16 ng/ml for 1).[11] In many cases, α-methylene carbonyl structures are responsible for cytotoxicity and other toxicities. In this case, however, it seems that the toxicity of these polyether compounds is not derived from the α-methylene carbonyl moiety. This observation is further corroborated with the report that the compound 2 also possesses ichthyotoxicity.[7]

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8. The strain used has the same origin (Wilson's collection) as those used by other researchers and has been maintained for 9 years in our laboratory. The mass culture was done in the artificial sea water NH-15 medium (modified by J. A. Gate and W. B. Wilson, Limnol. Oceanogr., 1960, 5, 171) under fluorescent light at 25°C without aeration.
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10. The 250 MHz ^{1}H-nmr spectrum, which was kindly supplied by Professor Nakanishi, Columbia University, was compared with our 500 MHz spectrum. Both spectra match in every detail except the signal δ1.30 (6H), which was resolved to two signals in the latter spectrum.
11. Minimum concentrations to cause 100% lethality to guppy fish, Poecilia reticulata within 24 hrs. Lin, et al. gave LC_{50}=16-25 ng/ml for 1 using Zebra fish, Brachydanio rerio (ref. 6 and M. Risk, K. Werrbach-Perez, J. R. Perez-Polo, H. Bunce, S. M. Ray, and J. L. Parmentier, In "Toxic Dinoflagellate Blooms," D. L. Taylor, H. H. Seliger, Eds., Elsevier-North Holland, New York, 1979, pp. 367-372). Baden, et al.[9] gave LC_{50}=11 ng/ml for his T_{34} mosquito fish, Gabusia affinis.

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