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THE STRUCTURE OF BREVETOXIN C

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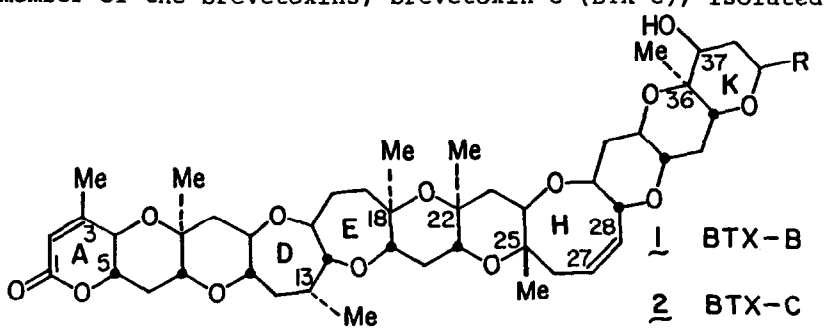
Abstract: The structure of brevetoxin C (BTX-C), a polyether marine toxin from Ptychodiscus brevis, has been elucidated by comparison of its spectral data with those of brevetoxin B (BTX-B).

Recently, we reported the structure of brevetoxin B (BTX-B) 1 isolated from the "red tide" dinoflagellate Ptychodiscus brevis which is responsible for massive fish kills along the Florida coast and in the Gulf of Mexico.[1] The structures of the brevetoxins represent a novel class of natural products characterized by long skeletons consisting of contiguous trans-fused ether rings. As opposed to tetrodotoxin and saxitoxin/gonyautoxin, the paralytic shell-fish poisons (PSP) which are water-soluble and block sodium channels[2], the brevetoxins are lipid-soluble neurotoxic shell-fish poisons (NSP) which activate sodium channels.[3,4] In the following we describe the structure of a second member of the brevetoxins, brevetoxin-C (BTX-C), isolated from the same source.



1 BTX-B R: $-CH_2-\overset{40}{C}(=CH_2)-\overset{42}{C}HO$

2 BTX-C R: $-CH_2-\overset{40}{C}(=O)-\overset{42}{C}H_2Cl$

Unialgal cultures of P. brevis were grown in artificial sea-water as previously described.[5] In a typical isolation, 50 L of medium (ca. 5 x 10^8 cells) was acidified to pH 5.5 and extracted with diethyl ether to give 90 mg of crude toxins. Flash chromatography of the mixture with 5% methanol in diisopropyl ether (v/v) gave 0.4 mg of BTX-C in addition to 0.8 mg of brevetoxin A (BTX-A) and 5.0 mg of BTX-B. The purity of isolated compounds was checked by high-performance LC, Whatman Partisil ODS-2 analytical column, MeOH-H_2O (4:1).

BTX-C exhibits the following physical constants: $C_{49}H_{69}ClO_{14}$; desorption/chemical ionization MS (D/CI-MS)[6,7,8] (Figure 1), m/z (relative intensity) 917 (100%) $[M+H]^+$, 934 (98%) $[M+NH_4]^+$; UV (MeOH) 208 nm (ε 11,300, $\pi\pi^*$ ene-lactone); CD (MeOH) 227 nm ($\Delta\varepsilon$ -2.53, $\pi\pi^*$ ene-lactone), 257 ($\Delta\varepsilon$ +5.55, $n\pi^*$ ene-lactone), 305 ($\Delta\varepsilon$ -0.12 $n\pi^*$ α-chloroketone); FTIR (KBr pellet) 1738 cm^{-1} (ene-lactone), 1729 (α-chloroketone).

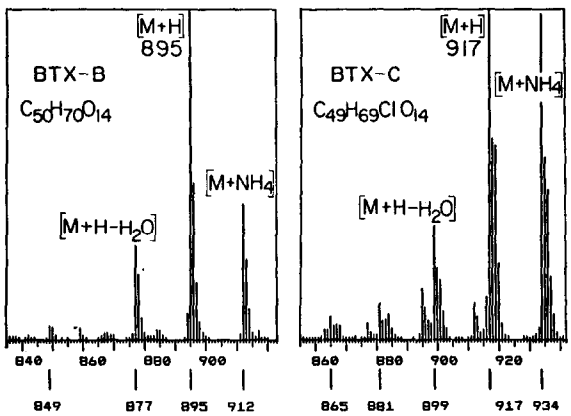


Figure 1. Desorption/chemical ionization mass spectra (molecular ion region) of BTX-B and BTX-C (NH_3 reactant gas).

A comparison of the quasi-molecular ion region of the D/CI-MS of BTX-C 2 with that of BTX-B[1] (Figure 1) shows a characteristic isotopic cluster in the $[M+H]^+$ and $[M+NH_4]^+$ peaks of BTX-C which are due to the presence of one chlorine atom. This is not observed in BTX-B ($C_{50}H_{70}O_{14}$).

The J-modulated spin echo ^{13}C-NMR[9,10] (62.9 MHz) of BTX-C (Figure 2), in which the quaternary and methylene carbons (even number of H's) appear as positive peaks whereas the methine and methyl carbons (odd number of H's) appear as negative peaks, disclose the presence of a saturated carbonyl at 201.0 ppm, ene-lactone carbons (ring A) at 163.6, 161.0 and 115.6 ppm, two =CH's (27,28-ene) at 135.1 and 127.2 ppm, five quaternary C-O's, seventeen CH-O's, thirteen CH_2's, one CH and seven CH_3's. The peaks are virtually superimposable with those of BTX-B 1 except for the absence of signals due to the enal moiety at 135.7 (C-43), 147.8 (C-41), and 194.2 ppm (C-42).

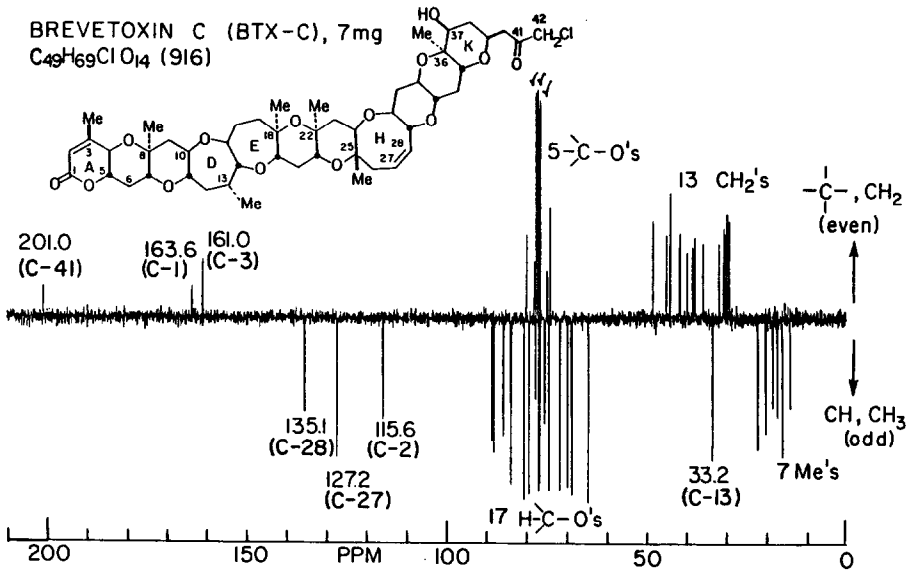


Figure 2. J-modulated spin echo ^{13}C-NMR (62.9 Mhz) of BTX-C.

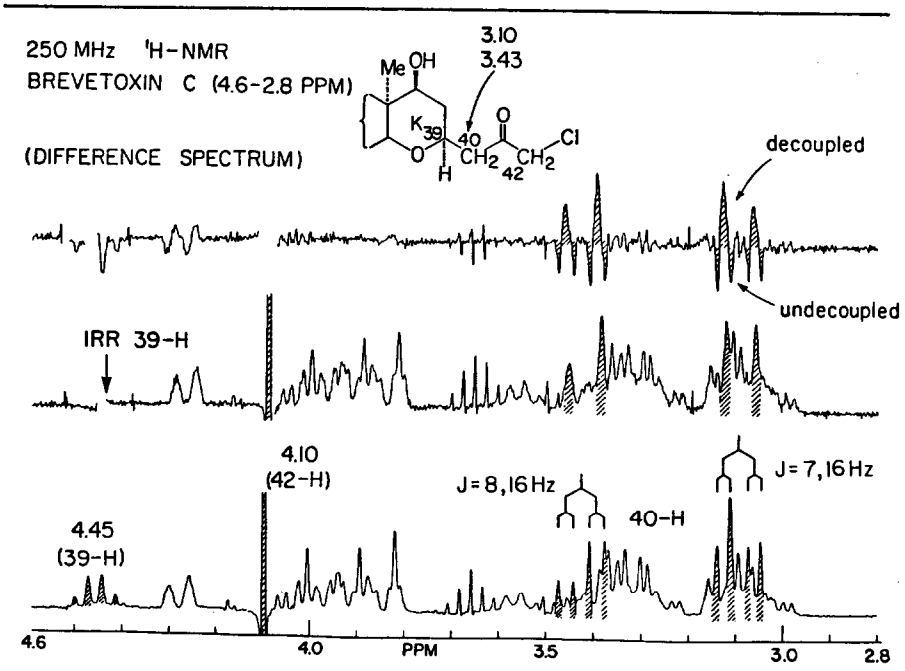


Figure 3. ^{1}H-NMR (250 MHz) decoupling difference spectrum of BTX-C.

This together with the presence of a saturated carbonyl (305 nm CD peak and 201 ppm ^{13}C-NMR signal) and a chlorine atom indicated that the structure of BTX-B and BTX-C differed only in the ring K side chain. This conclusion is

supported by comparison of the high field regions of the ^{13}C-NMR spectra where the 31.8 ppm peak (C-40) in BTX-B 1 is replaced by two new signals at 43.8 ppm (C-40) and 48.4 ppm (C-42) in the spectrum of BTX-C 2. It is readily seen in the ^{1}H-NMR decoupling difference spectrum (Figure 3) that irradiation of the 39-H proton causes the 40-H protons to collapse into AB doublets. Thus the side-chain of BTX-C is $-CH_2COCH_2Cl$ as shown in structure 2.

Due to attempts by different groups to isolate these toxins, the nomenclature has been confusing. The situation can now be summarized as follows (private communication, Professor Y. Shimizu and direct comparisons): GB-2 isolated by M. Alam and Y. Shimizu[11] and T34 isolated by D. G. Baden et. al.[12] correspond to BTX-B. The structure of the most active and elusive toxin, BTX-A, is currently under investigation in our laboratory.

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References and Notes

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