

Isolation, purification, structural determination, and monitoring of Goniodomin A produced by the red tide-associated dinoflagellate, *Alexandrium monilatum*

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Abstract.

The chain-forming dinoflagellate *Alexandrium monilatum*, formerly known as *Gonyaulax*, was first observed in 1936 on the Texas coast in Offats Bayou where it caused annual formation of red colored water, as well as severe fish mortality. According to Connell and Cross, local residents of that area observed red water and dead fish virtually every year 15-20 years prior to their 1949 report. It has also been reported to be associated with widespread discolored water and increased fish mortality in the Mississippi Sound, off the eastern and western coasts of Florida, and in the Gulf of Nicoya, Costa Rica. Early studies have found that *A. monilatum* produced a harmful substance(s) that is predominantly contained in the cell mass and increases toxicity when the organism cytolyzes. Our studies corroborate with other research findings demonstrating that the toxin has low water solubility, casting doubt on the presence of more typical water soluble saxitoxin-like toxins that are water soluble. Using sophisticated chemical, chromatographic, and analytical chemistry techniques, we have successfully purified and identified the molecular structure of the toxin produced by *A. monilatum*. To solve the molecular structure of the toxin, we utilized a 500MHz NMR equipped with the following experiments: ¹H, ¹³C, COSY, HSQC, HMBC. In addition, mass analysis utilized ESI-MS, MALDI-TOF MS, and Q-TOF MS. The toxin represents a polyether macrolide with an empirical formula of C₄₃H₆₀O₁₂. This toxic compound is identified as Goniodomin A, identical to the one produced by the rock pool-blooming dinoflagellate, *Alexandrium pseudogoniaulax*. This compound had not been previously isolated from *A. monilatum*. We have successfully solved the structure of a toxin that has caused fish mortalities in the Gulf of Mexico for over 60 years.

Current studies into environmental impacts and toxin detection/monitoring are underway. We have established mass spectrometric standard curve using our own internal standards for the toxin. This method will now be used to monitor toxin production over the growth cycle of the dinoflagellate. This will provide information regarding any variances in toxin production over the growth of the dinoflagellate.

Funding provided by NOS/NOAA.