海産ラン藻 Okeania hirsuta 由来の低分子化合物群に関する研究

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract of Dissertation

専　攻 Major	応用環境システム学	氏　名 Name	ZHANG BOTAO
論文題目 Title	Secondary metabolites from the marine cyanobacterium *Okeania hirsuta*: chemical and biological aspects		

Filamentous benthic marine cyanobacteria from the tropics have been a prolific source of structurally unique bioactive secondary metabolites. Among them, cyanobacteria ascribed to the genus *Lyngbya* were recognized as a potential therapeutic gold mine, rich in structurally diverse and highly bioactive natural products. However, recent phylogenetic analyses have led to the taxonomy revision on this genus, resulting in the establishment of six new cyanobacterial genera from the *Lyngbya* including genera *Dapis*, *Limnoraphis*, *Moorena* (previously *Moorea*), *Microseira* and *Okeania*.

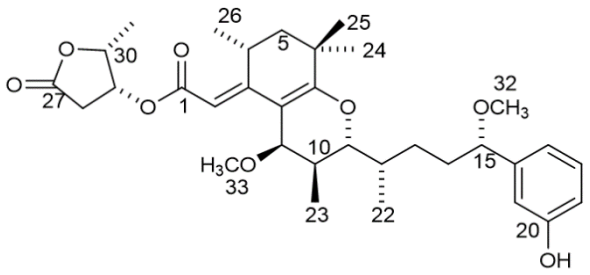
In July 2010, a massive outbreak of toxic filamentous cyanobacteria occurred in Okinawa prefecture. The predominant species in this sample was later identified as *Okeania hirsuta* by an analysis of the 16S rRNA sequence. Because of this cyanobacterial boom, we were able to collect a large number of samples, nearly 20 kg, and conduct a comprehensive investigation of the bioactive components in them. Throughout our extensive exploration on this *O.hirsuta*, the primary toxic constituents were uncovered as aplysiatoxin (ATX)-related compounds. Furthermore, a variety of secondary metabolites belonging to different class have been discovered, highlighting the exceptional biosynthetic capability of this sample. Regarding this remarkable biosynthetic ability and the substantial quantity of the sample obtained, we attempted to analyze and characterize the trace components from unpurified subfractions of this sample, including side fractions, aiming to discover structurally novel and bioactive compounds. In addition, the biosynthetic gene cluster and pathway for ATX remain unknown. We are eager to find more new natural analogs and ATX-related compounds in this sample to gain insights into their biosynthetic mechanisms.

1. ATX-related compounds (ATXs) from Okinawan cyanobacterium *Okeania.hirsuta*

The ethyl acetate extracts of this *O. hirusuta* sample afford a number of ATX derivatives, including a new compound debromooscillatoxin G.

Debromooscillatoxin G

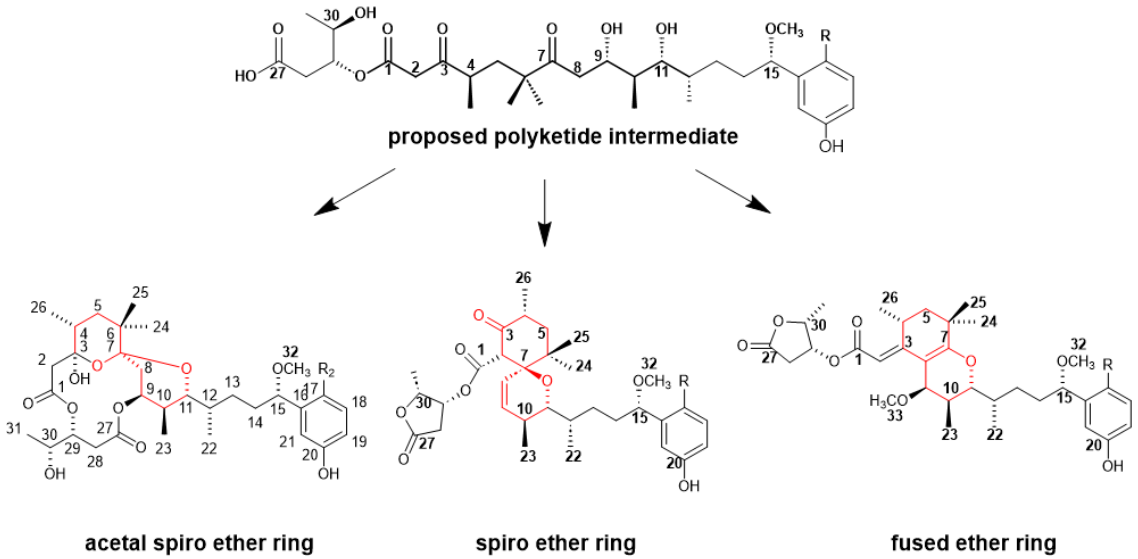
Debromooscillatoxin G was obtained as a white amorphous solid. Its molecular formula of $C_{33}H_{46}O_8$ with 11 degrees of unsaturation was deduced from a prominent $[M+H]^+$ peak at *m/z* 571.3288 (calcd. for $C_{33}H_{47}O_8$: *m/z* 571.3265). The existence of the phenol side chain (C-12 to C-22) and γ-lactone ring were confirmed by COSY and HMBC correlations, together with the comparison of proton chemical shifts with those of 30-Me-OTX D. Furthermore, the observed unsaturation and HMBC correlations revealed that phenol side chain and γ-lactone ring were connected by an oxabicyclo[4.4.0]decane structure. The relative configuration of debromooscillatoxin G was established by the NOESY correlations and the comparison of vicinal coupling constants with those of 30-Me-OTX D.



debromooscillatoxin G

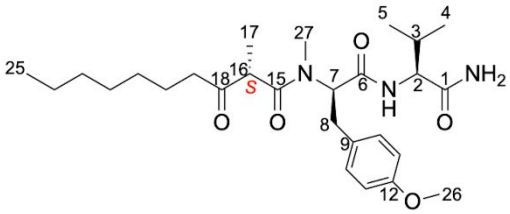
Putative biosynthetic pathways for ATXs

Overall, 24 ATX-related compounds were isolated from the Okinawan cyanobacterium *O. hirsuta* by our laboratory. Based on the structures of these isolated compounds, a common polyketide intermediate was deduced. From this intermediate, three potential biosynthetic pathways were proposed according to the formation of the ring system.

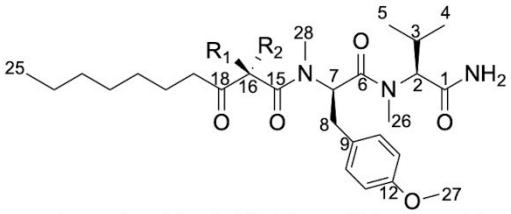


2. *N*-Desmethylmajusculamide B, a Lipopeptide from the Okinawan Cyanobacterium *Okeania hirsuta*

A new lipopeptide, *N*-desmethylmajusculamide B (**1**), together with two known compounds majusculamide A (**2**) and majusculamide B (**3**), was isolated from the cyanobacterium *Okeania hirsuta* from Okinawa, Japan. The absolute configurations of the amino acid residues were determined using Marfey's method. Furthermore, a semi-synthesis of *N*-desmethylmajusculamide B from **3** was conducted to establish the absolute configuration of C-16 as *S*. In the cytotoxicity test against mouse L1210 leukemia cells, the activities of **1** and **3** were almost identical. In terms of structure-activity relationship, the presence of a methyl group on *N*-methylvaline in majusculamides appears to be less important for their bioactivities. In addition, the cytotoxicity of **2** was more potent than those of **1** and **3**. In this regard, the configuration of the C-16 position was again shown to have a significant impact on biological activity.



N-desmethylmajusculamide B (**1**)



majusculamide A (**2**): $R_1 = CH_3$, $R_2 = H$
majusculamide B (**3**): $R_1 = H$, $R_2 = CH_3$

In summary, this study demonstrates that marine cyanobacteria are suitable sources for the discovery of trace compounds with novel chemical structures and biological properties. Meanwhile, the quantity of sample is very crucial in such explorations.