



HOKKAIDO UNIVERSITY

Title	New secondary metabolites from marine cyanobacteria of the genus Moorea collected in Malaysia and Saudi Arabia [an abstract of dissertation and a summary of dissertation review]
Author(s)	JULIUS ADAM, VELASCO LOPEZ
Degree Grantor	北海道大学
Degree Name	博士(環境科学)
Dissertation Number	甲第12488号
Issue Date	2016-12-26
Doc URL	https://hdl.handle.net/2115/64733
Rights(URL)	https://creativecommons.org/licenses/by-nc-sa/2.1/jp/
Type	doctoral thesis
File Information	Lopez_Julius Adam Velasco_abstract.pdf, 論文内容の要旨



学 位 論 文 内 容 の 要 旨

博士 (環境科学)

氏 名 LOPEZ JULIUS ADAM VELASCO

New secondary metabolites from marine cyanobacteria of the genus *Moorea* collected in Malaysia and Saudi Arabia

(マレーシアおよびサウジアラビア由来海洋ラン藻*Moorea*属から得られた
新規2次代謝産物)

Marine natural products research provides insight into the chemistry of the subject organisms and their communities, as well as their ecological significance. Among these organisms are the marine cyanobacteria, which have been a consistent source of significant bioactive compounds with unprecedented structures. The objective of this study is to discover novel bioactive compounds from cyanobacteria collected from understudied maritime areas. It is projected that the research data will also provide knowledge on the biodiversity and underlying chemistry involved in the target organisms and their habitat.

A total of 44 marine cyanobacterial samples from Malaysia and Saudi Arabia were subjected to liquid chromatography-mass spectrometry (LC/MS) profiling and cytotoxicity screening against human MCF7 breast cancer cells. By using 16S rRNA gene sequencing analysis, nine samples from Saudi Arabia were identified as *Moorea producens* (2), *Moorea bouillonii* (1), *Symploca* sp. (1), *Trichodesmium erythraeum* (1), and *Okeania* sp. (1) or related to *Okeania* sp. (3), while 11 samples from Malaysia were found to be *Caldora penicillata* (1) and *M. bouillonii* (10). Eleven of these identified samples showed potent cytotoxicity at 1 µg/mL. The dereplication of known compounds was performed by using the MarinLit database. Following an MS-guided approach, two cytotoxic samples were pursued to isolate potential novel compounds.

The lipophilic fraction of an *M. bouillonii* sample from Malaysia exhibited di- and trichlorinated compounds in its mass spectrum. This was subjected to silica gel column chromatography, solid-phase extraction, and high performance liquid chromatography (HPLC) to yield three known cytotoxic compounds – apratoxins A and C, wewakazole; and two new acyl amides – columbamides D and E, which are di- and trichlorinated, respectively. The structures were elucidated by using a combination of MS and nuclear magnetic resonance (NMR) techniques. The molecular formulas were deduced based on high resolution (HR) MS data. The 2D NMR data were used to assemble the structure. In particular, the heteronuclear 2-bond correlation (H2BC) technique was useful in positioning the chloromethine in the long chain alkyl group. Meanwhile, nondecoupled heteronuclear single quantum correlation (HSQC) data revealed the *E* geometry of

the olefin ($^3J_{\text{H,H}} = 16 \text{ Hz}$). The partial synthesis of the *N,O*-dimethylserinol was carried out and the synthetic products were used in Marfey's analysis to conclude an *R* configuration. Subsequently, total synthesis of two diastereomers (10*S*,20*R* and 10*S*,20*S*) of columbamide D were achieved. By comparison of the ^1H and ^{13}C NMR chemical shifts and results of the Marfey's analysis of the synthetic versus the natural compounds, columbamide D and E were concluded to have the 10*S*,20*R* configuration.

The second sample was *M. producens* from Saudi Arabia. A possible new compound with $[\text{M}+\text{H}]^+ m/z 1127$ was found in the EtOAc fraction and was targeted for purification. The sample was subjected to silica gel column chromatography and a series of HPLC to yield the known cytotoxic compound curacin D, and the new cytotoxic cyanobactin, wewakazole B. The structure elucidation was carried out by a combination of NMR, MS, and MS/MS techniques. The molecular formula was deduced based on HRMS data. Nine common and three modified amino acid residues were assembled from the 2D NMR data. The cyclic structure was supported by the number of unsaturations and MS/MS data. After ozonolysis and acid hydrolysis, the absolute configurations of the amino acid residues were determined to be L by chiral-phase LC/MS and HPLC analyses. Wewakazole B was cytotoxic to MCF7 cancer cells ($\text{IC}_{50} = 0.58 \text{ }\mu\text{M}$) and to human H460 lung cancer cells ($\text{IC}_{50} = 1.0 \text{ }\mu\text{M}$).

By using an LC/MS-based approach, the chemical profiles of different marine cyanobacterial samples from Malaysia and Saudi Arabia were analyzed against the MarinLit database. This strategy efficiently dereplicated several known compounds such as apratoxins, dolastatins, and lyngbyabellins. Thus, the investigator was able to save time and resources by having clear targets and focusing on them through MS. This led to the successful isolation of three new compounds. The results also show the potential of marine cyanobacteria as a source of cytotoxic compounds. The wewakazoles showed moderate cytotoxicity, while the synthesized columbamide D stereoisomers did not show potent cytotoxicity. The high cytotoxicity observed in the Malaysian *M. bouillonii* was most likely due to the apratoxins, which are cytotoxic at nanomolar levels. This study was also able to confirm that the wewakazoles are not siderophores as previously predicted.

Furthermore, the 16S rRNA gene and MS data showed the difference in cyanobacteria biodiversity between the Red Sea and South China Sea. It is noteworthy that even without 16S rRNA gene data, the samples could be differentiated based on their chemical profiles. The compounds found in a certain species may serve as chemotaxonomic markers for easy identification. Finally, it is concluded that the genus *Moorea* remains to be a promising source of new bioactive compounds and an attractive subject for drug discovery.