博士論文（要約）

Investigation on new bioactive peptides from marine invertebrates

(海洋無脊椎動物由来新規生物活性ペプチドに関する研究)

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[Introduction]

Through the years, marine invertebrates have been a source of new and bioactive molecules, making them an attractive source of natural products and drug leads. Furthermore, they are known to host symbionts that possess the genetic ability to code for these bioactive molecules. In this work, chemical investigations of the scarcely-studied side-gilled sea slug, *Pleurobranchus forskalii*, and the marine sponge, *Discodermia kiiensis* were conducted. Structurally complex and bioactive peptidic molecules were discovered, and their complete chemical structures were successfully elucidated in this study. These findings pave the way to the discovery of the putative gene clusters encoding these structurally interesting, and bioactive drug leads.

[Results and Discussion]

1. Ergosinine (1) and Cycloforskamide (2) from *P. forskalii*

The chloroform extract of *P. forskalii* afforded an ergot peptide alkaloid, ergosinine (**1**), and a novel macrocyclic cytotoxic dodecapeptide, cycloforskamide (**2**) (**Figure 1**).

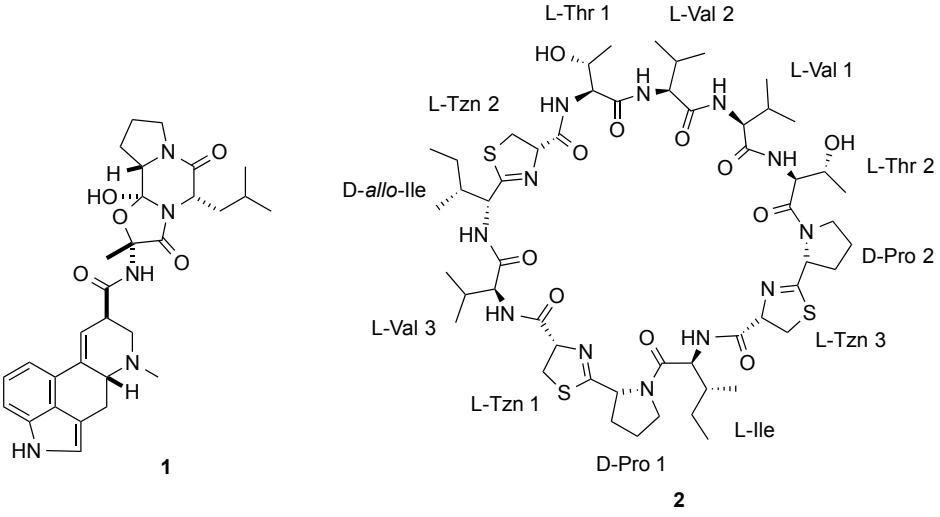

Figure 1. Bioactive compounds isolated from *P. forskalii*

The structure of **1** was confirmed by spectral analysis, and its absolute configuration by optical rotation. Although ergot alkaloids have been widely isolated from terrestrial higher plants and fungi, this is the first report of isolating an ergopeptine from marine life. This finding expands the known extent of geographical distribution of ergot alkaloids from terrestrial to aquatic life.[1] Meanwhile, the planar structure of **2** was deduced by extensive NMR analyses, and was further confirmed by MS/MS fragmentation analyses. Finally, the absolute configuration was determined by total

hydrolysis and chiral-phase GC-MS analysis. This novel dodecapeptide contains three D-amino acids and three thiazoline heterocycles, and exhibits cytotoxicity against P388 cells, with an IC_{50} of 6 μM.[2]

2. Lipodiscamides A-C (3-5) and Sulfolipodiscamides A-I (6-14) from *D. kiiensis*

Lipodiscamides A-C (**3**-**5**), three new lipodepsipeptides, were characterized from the ether fraction of *D. kiiensis* (**Figure 2**). These structurally rare cyclic lipodepsipeptides were found to possess an unprecedented dilactone macrocycle, and thus represent a new family of lipopeptides. They are the only lipopeptides bearing 4*S*-hydroxy-*trans*-2-enoate, and non-canonical amino acids, L-3-ureidoalanine (Uda), *E*-dehydronorvaline (Denor), and D-citrulline (Cit). Their absolute stereochemistry was elucidated by chemical degradation and derivatization of **3**-**5**, synthesis of authentic standards, and chiral-phase GC/MS analysis.

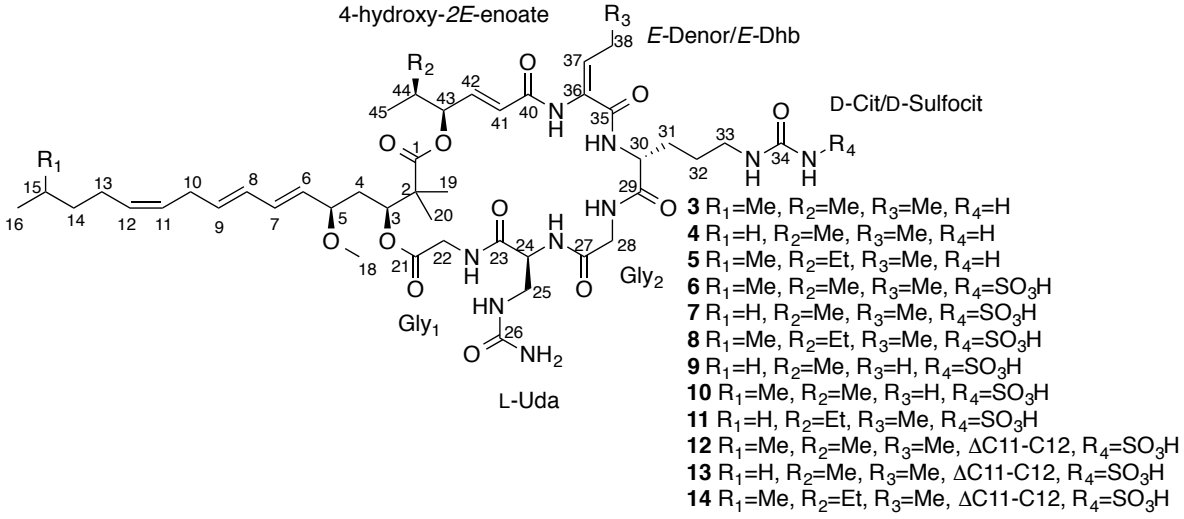

Figure 2. New compounds characterized from *D. kiiensis*

MTT assays against P388 and HeLa cells revealed the moderate cytotoxicity of all three compounds.[3] Furthermore, *N*-sulfoureidylated analogues, sulfolipodiscamides A-I (**6**-**14**), were isolated by gel filtration chromatography of the *n*-butanol fraction of *D. kiiensis*. By extensive NMR analyses, the structures of **6**-**14** were elucidated as having a sulfonate on the ureido group of the D-citrulline residue. This sulfoureido functionality was found to be acid-labile, and remarkably, its occurrence has never been documented in nature.[4] For the minor metabolites, an *E*-Dhb residue replaced *E*-Denor in **9**-**10**, while

the C11-C12 unsaturation is reduced in **12**-**14**. The absolute stereochemistry of **6**-**8** was confirmed by comparison of HPLC retention times of the hydrolytic product and the corresponding authentic lipodiscamide. Interestingly, sulfolipodiscamide A displayed a 2.3-fold increase in cytotoxicity against murine leukemia (P388) cells, compared to the unconjugated parent compound.

[Overall Conclusion]

Cytotoxic and novel peptidic metabolites were characterized from both marine invertebrates investigated, validating their high proficiency as source of bioactive and structurally interesting marine natural products that could serve as lead compounds for drug development. Therefore, the results of this study are worth of PhD thesis of the University of Tokyo.

References

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2. Tan, K., Wakimoto, T., Takada, K., Ohtsuki, T., Uchiyama, N., Goda, Y., Abe, I. *J. Nat. Prod*. **2013,** *76 (7),* 1388-1391.
3. Tan, K., Wakimoto, T., Abe, I. *Org. Lett*. **2014,** *16,* 3256-3259.
4. Tan, K., Wakimoto, T., Abe, I. **2016,** Submitted for publication.