論文内容の要旨

水圏生物科学
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Studies on cell differentiation inducers and cytotoxic compounds from marine invertebrates
（海産無脊椎動物由来の細胞分化誘導および細胞毒性物質に関する研究）

Marine invertebrates such as sponges, jellyfish, soft corals, sea hares are exceptional sources of secondary metabolites. These natural products have unique biological activities and diverse chemical structures which provide a promising approach to the development of chemotherapeutic agents. Therefore, scientists all around the world have isolated biologically active compounds and determine their structures. Among them, cytotoxic compounds have a prominent role in the medical treatment of cancer; In vivo and in vitro analyses have revealed that several type of compounds isolated from marine organisms developed a cytotoxic behavior towards different type of cancer cell lines. Indeed, four cytotoxic compounds derived from marine natural products have been approved for anti-cancer drug. Several compounds or their synthetic derivatives are under clinical trials. The use of anti-cancer drugs together with radiation the

rapy depends on the destruction of neoplastic cell, which their repercussion is the slow growth and damage of healthy cells. For that reason and in order to avoid the side effects in the condition of the patient, less invasive therapies should be consider as future treatments. One of them is the cell differentiation induction of cancer cells in which a compound induces mature cancer cells into normal cells. This is a hopeful treatment because cell differentiation inducers do not rely on cytotoxicity. An excellent example of differentiation into normal cells is the treatment of leukemia cancer cell line K562. This cancer cell was established from a patient with chronic mylogenous leukemia in terminal blast crisis. K562 cells by chemical stimuli such as doxorubicin or aphidicolin can differentiate into erythrocytes, megakaryocytes and granulocytic cells.

In the early stage of this study, I focused on compounds exhibiting cytotoxicity from marine invertebrates. As a result of screening of the extracts showing cytotoxicity against HeLa cells, a non-identified marine sponge was selected for further research. The sponge was blended and the extract was subjected to solvent partitioning and ODS flash chromatography followed of bioassay-guided fractionation including RP-HPLC to afford the bioactive compounds. New polyacetylene **1** was isolated together with known metabolites petrosiacetylene D and penasterone (**Figure 1**).

Interpretation of the NMR data of **1** revealed the presence of one acetylenic proton, one

oxygenated methines and a non-identified number of methylenes. Two partials structures (A and B) were determined by ^{1}H-^{1}H COSY and HMBC correlations. Partial structure A, characteristic of the terminal structure of sponge-derived acetylenes, and the geometry of the double bond was determined *E* based on the vicinal coupling constant (18 Hz). Partial structure B was composed by two doubled bonds with *Z* geometry determined by the ^{13}C-NMR chemical shifts of the allylic carbons. These double bonds were separated by one methylene carbon. The chemical structure of **1** resemble to the one of the durynes.

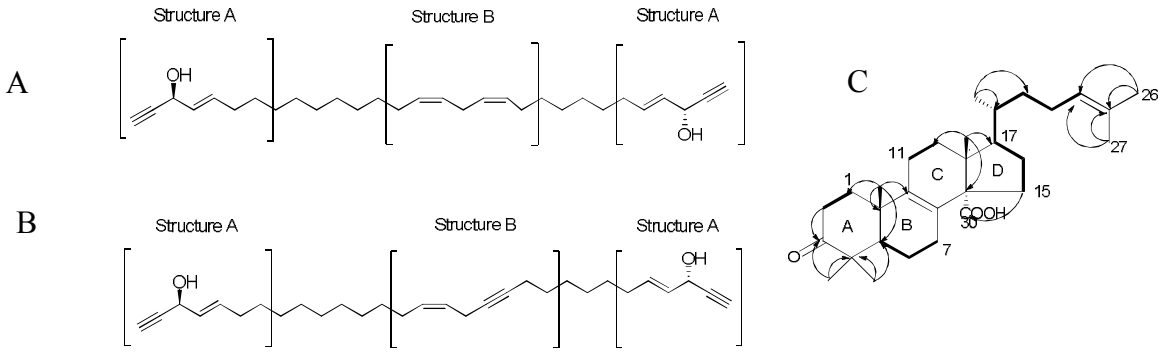

Figure 1. Chemical structure of A) 1, B) petrosiacetylene D and C) penasterone

In order to determine the exact number of the methylenes and the location of the two double bonds, tandem-FABMS analysis and ozonolysis reaction were conducted. After the analyses, the locations of the double bonds were determined to be C-13 and C-16 and the resulting length of the methylene chain (**Figure 1**). Following the bioassay-guided fractionation of the extract, the known compounds petrosiacetylene D and penasterone were isolated. Their chemical structures were elucidated by 2D-NMR and tandem FAB-MS analyses and chemical methods including ozonolysis and the modified Mosher`s method.

In the screening, it was observed that the aqueous fraction of a zoanthids specimen had a potent cytotoxicity. Therefore, we conducted further analysis to isolate cytotoxic constituents. LC-MS analysis of the bioactive fractions separated by RP-HPLC showed an ion peak (*m/z* 1340 $[M+2H]^{2+}$) which is the same molecular weight of cytotoxic palytoxin (**Figure 2**). These results suggested that the cytotoxic compound is palytoxin.

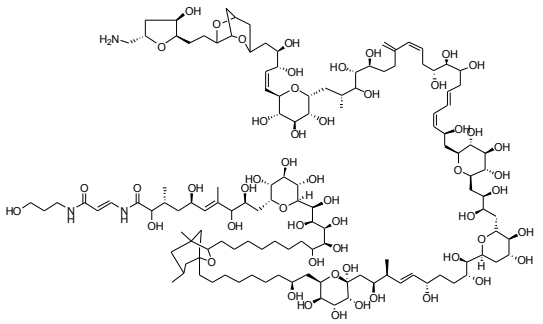

Figure 2. Chemical structure of palytoxin.

In the later stage of my investigation, I decided to search for secondary metabolites which possess a more specific biological activity, and I conducted a screening assay for cell differentiation inducers of leukemia cancer cell K562. The extract of a marine sponge *Biemna sp.* exhibited significant cell differentiation of K562 into erythrocytes. The sponge was extracted with MeOH and the extract was partitioned between $CHCl_3$ and H_2O. The organic fraction was further partitioned between *n*-hexane and 90% MeOH. The bioassay-guided fractionation of the 90% MeOH fraction by ODS flash chromatography and RP-HPLC afforded two new pyridoacridines, *n*-hydroxymethylisocystodamine and neolabuanine A, as well as the previously reported natural products ecionine A, ecionine B, isocystodamine, *n*-methylisocystodamine, 9-hydroxyisoascididemin and bienmadin (**Figure 3**).

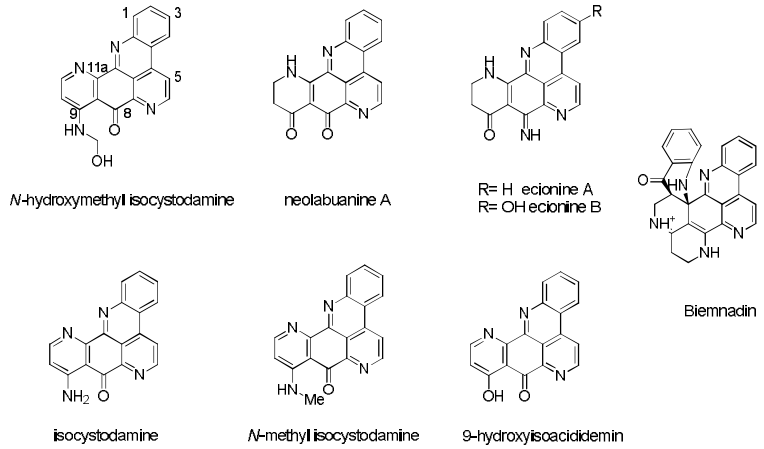

Figure 3. Chemical structure of the pyridoacridine compounds isolated from*Biemna sp*.

The structures of the pyridoacridines were determined by 2D NMR spectroscopy and LC-MS data.^{1}H-^{1}H coupling constant, characteristic carbon chemical shifts, and HMBC correlations suggested the presence of one disubstituted benzene ring and two trisubstituted pyridine ring, in *N*-hydroxymethylisocystodamine which are closely related with those of isocystodamine. However, the primary amino group found in isocystodamine was substituted by a hydroxymethyl group in *N*-hydroxymethylisocystodamine. LC-MS data showed that this compound graduallytransforrmed into isocystodamine. NMR data of neolabuanine A revealed that the structure of neolabuanine A was identical with that of reported labuanine A, and the compound labuanine A isolated by Aoiki *et al*. as was ecionine A.

Spontaneous chemical transformations were observed in the isolated pyridoacridines, LC-MS data showed that ecionine A gradually converted into 9-hydroxyisoascididemin and its tautomeric forms. Furthermore, cystodimine A, another pyridoacridine with the same molecular weight and a linear distribution of its rings system instead of angular type of ring system in ecionine A, showed similar chemical transformation as that of ecionine A (**Figure 4**). These analyses suggested that these compounds are precursors of other pyridoacridines. All the compounds showed cell differentiation induction of K562 cells to mature erythrocytes with exception of 11-hydroxyascididemin. The induction to erythrocytes was determined by chemical staining and microscopic observation.

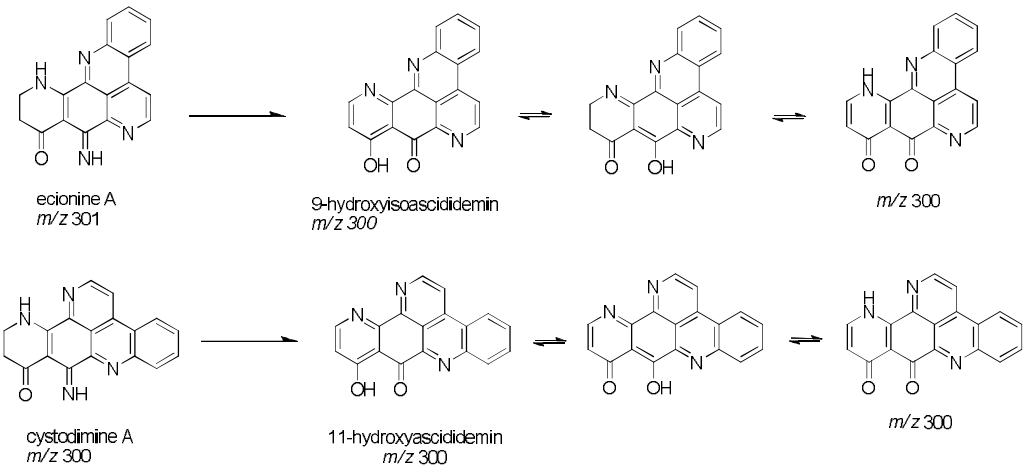

Figure 4.

Ecionine A and cystodimine A and their chemical transformations.

Lastly, two more sponges were selected for further researches because they also induced cell differentiation of K562 cells into erythrocytes. Bioassay-guided fractionations and LC-MS analysis afforded an active compound that seemed to be swinholide A. In order to analyses the nature of this compound, RP-HPLC purification was conducted the ^{1}H NMR spectrum of the active metabolite as swinholide A itself.

The variety of biological activities against cancer cells induced by marine natural products was confirmed by the isolation of cytotoxic compounds against HeLa cells and cell differentiation inducers of K562 cells. The chemical diversity of the compounds isolated from sponges, like the case of polyacetylenes, triterpenoids and the family of pyridoacridines, could prove that the extract of marine invertebrates can be used for the further development of modern drugs. Most of the pyridoacridines isolated showed in this study promising activity in K562 cells. Particularly pyridoacridines with the angular distribution of their rings showed more potent cell differentiation that with linear-type ring systems. Together these characteristic structural features could provide future insights into how this family of compounds can induce specific cell differentiation in K562 cells.