報告番号	※　　第　　号

主　論　文　の　要　旨

論文題目　Chemical studies on antifungal cyclic depsipeptides from an aquatic hyphomycete
（水生不完全菌由来の抗真菌性環状デプシペプチドに関する化学的研究）

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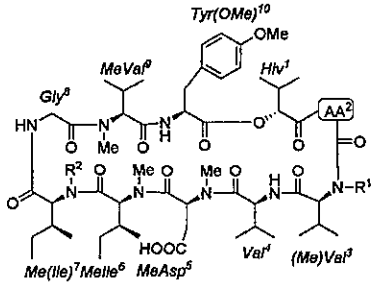
論　文　内　容　の　要　旨

Aquatic hyphomycetes are fungi occurring mainly in lotic fresh waters. They, unlike actinomycetes and other terrestrial fungi, release a vast number of asexual spores with characteristic shapes such as sigmoid or multiradiate forms and represent unexplored metabolites. The antifungal cyclic depsipeptides clavariopsins A and B (1 and 2) were isolated from the aquatic hyphomycete *Clavariopsis aquatica* in 2001 in my laboratory. In this thesis, I tried to isolate further related constituents from *C. aquatica* in a large scale and investigated their structure analysis and biological evaluation. The synthesis of some analogs of clavariopsin A (1) and their biological evaluations were also discussed in this thesis.

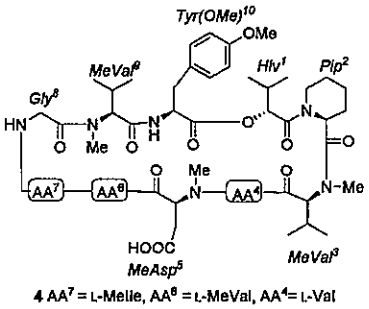
The culture broths (15 L in total, 100 flasks) were extracted with acetone, and the extract was partitioned between EtOAc and H_2O. The EtOAc fraction was separated by silica gel column ($CHCl_3$-MeOH), then cyclic peptide fractions were purified by column chromatography followed by repeated preparative HPLC. As a result, seven new cyclic depsipeptides, clavariopsins C–I (3–9), together with two known congeners, clavariopsins A and B (1 and 2), were isolated. The planer structures of isolated compounds were determined by spectroscopic analyses (NMR and MS) and the stereochemistry was confirmed by LC/MS (advanced Marfey's method) and chiral-phase HPLC as shown in Figure. The structures are different from 1 in *N*-methylation position or one amino acid moiety. Their antifungal and cytotoxic activities were evaluated against six important plant pathogenic fungi (*Botrytis cinerea, Magnaporthe oryzae, Colletotrichum orbiculare, Fusarium oxysporum, Alternaria alternata, and Aspergillus niger*) by paper disk diffusion

method and a cancer cell line (HeLa-S3) by MTT assay, respectively. Majority of the compounds exhibited potent antifungal activity against the fungi tested (minimum inhibition dose = 0.01–10 μg/disk) and induced hyphal swelling in *A. niger* (minimum effective dose = 0.3–3 μg/disk). The hyphal malformation induced in *A. niger* suggests that the clavariopsins may inhibit cell wall (1,3-glucan, etc.) biosynthesis of the fungi. Since the clavariopsins exhibited low cytotoxicity towards the cancer cell line (IC_{50} > 10 μM), these compounds could be a promising class of antifungal agents.

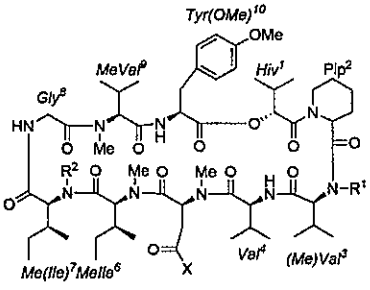
To develop effective bioactive agents by varying cell permeability, the most abundant congener clavariopsin A (**1**) was converted to methyl ester **10** and amide derivatives **11a–d** (Figure) by modifying the *N*-methylaspartic acid ($MeAsp^5$) residue. They showed significant decrease of the antifungal activity (minimum inhibition dose = 0.1– >10 μg/disk), demonstrating an important role of the carboxy group of $MeAsp^5$. Their cytotoxicity against HeLa-S3 cells was also evaluated. Interestingly, the primary amide **11a** exhibited a higher cytotoxicity (IC_{50} = 10 μM) than that of the parent natural compound **1** (IC_{50} = 20 μM). Others (**10**, **11b–d**) displayed lower cytotoxicity or inactive (IC_{50} = 19 or >50 μM), which is in a negative correlation against the molecular lipophilicity. This information would be useful for further optimization of the clavariopsins, in which both the molecular lipophilicity and the position of chemical modification should be considered for developing more potent antifungal and less-cytotoxic agents.

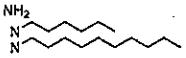
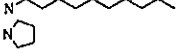


1 AA^2 = L-Pip, R^1 = Me, R^2 = Me
2 AA^2 = L-Pip, R^1 = H, R^2 = Me
3 AA^2 = L-Pip, R^1 = Me, R^2 = H
8 AA^2 = L-Pro, R^1 = Me, R^2 = Me



4 AA^7 = L-MeIle, AA^6 = L-MeVal, AA^4 = L-Val
5 AA^7 = L-MeVal, AA^6 = L-MeIle, AA^4 = L-Val
6 AA^7 = L-MeIle, AA^6 = L-MeLeu, AA^4 = L-Val
7 AA^7 = L-MeLeu, AA^6 = L-MeIle, AA^4 = L-Val
9 AA^7 = L-MeIle, AA^6 = L-MeIle, AA^4 = L-Leu



10 X = OMe
11a X = NH_2
11b X = 
11c X = 
11d X =