Bioorganic Chemical Studies on Wild Edible Mushrooms

学 位 論 文 要 約

Summary of Doctoral Thesis

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論文題目：野生の食用キノコに関する生物有機化学的研究
Title of Thesis：Bioorganic Chemical Studies on Wild Edible Mushrooms

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Summary：

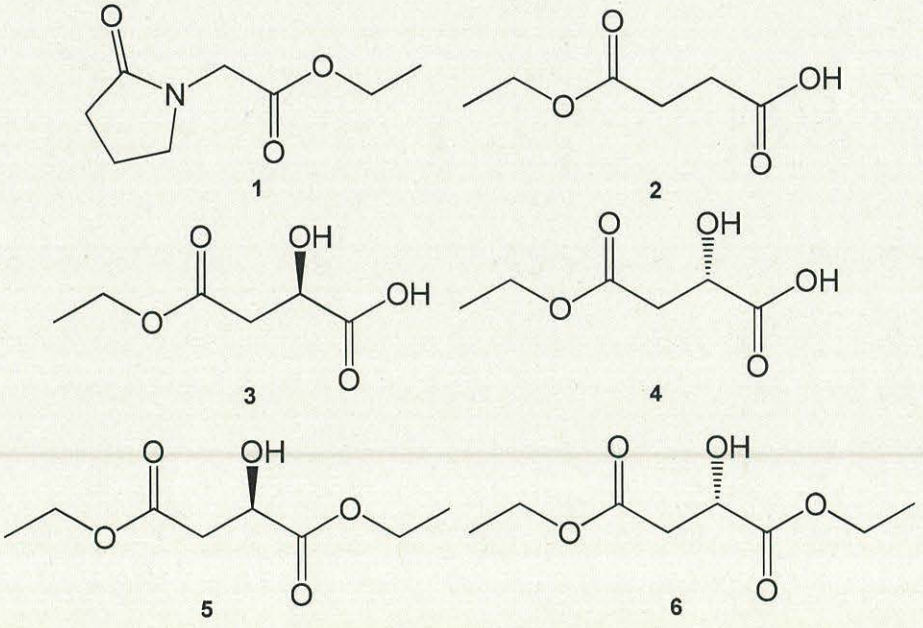
Wild edible mushrooms are widely accepted as food and generally consumed worldwide as medicinal products. They are becoming essential in the human diet for their nutritional and pharmacological roles. The effort to find secondary metabolites with significant activity has attracted attention of scientist. Research into chemical composition and biological properties of the wild mushrooms has been widely published. The chemical investigation of fruiting bodies as well as mycelia of higher fungi to discover new compounds along with their bioactivities is carried out extensively. In the previous study, Search for bioactive metabolites from three kinds of mushrooms *Leucopaxillus giganteus*, *Stropharia rugosoannulata*, and *Entoloma clypeatum* were carried out.

1) Bioorganic chemical studies on wild edible mushroom *Leucopaxillus giganteus*

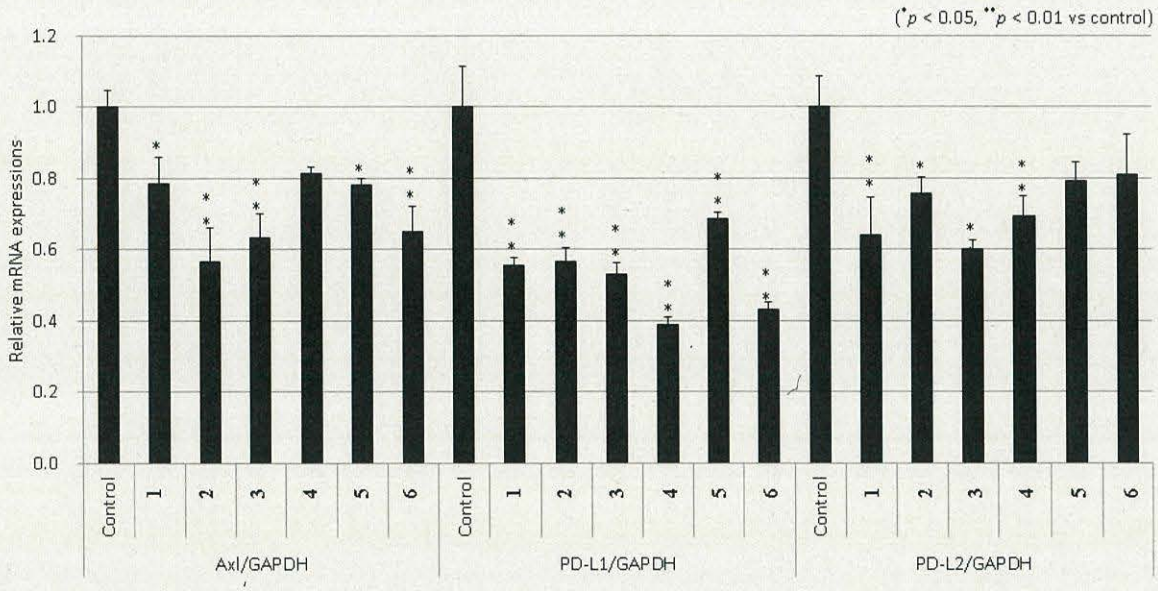
L. giganteus is a wild edible mushroom which belongs to *Tricholomataceae* Family commonly called giant funnel mushroom in English or Ooichotake in Japanese. Evaluation of chemical constituents and nutritional values of *Leucopaxillus giganteus* have been considerably studied. In this study, the chemical exploration of mushroom *L.*

giganteus was performed along with Axl and immune checkpoint suppression activities and plant growth regulating activity. Fresh fruiting bodies of *L. giganteus* was extracted with EtOH and then acetone to obtain the crude extracts. The partition method divided them into *n*-hexane, EtOAc, and EtOH soluble parts. The EtOAc soluble part was subjected to silica gel flash column chromatography, which was further purified by HPLC. Structural identification of each compounds was performed by the interpretation of spectroscopic data analysis. As a result, three compounds were isolated and identified as ethyl 2-(2-oxopyrrolidin-1-yl)acetate (**1**), monoethyl succinate (**2**), and 4-ethoxy-2-hydroxy-4-oxobutanoic acid (**3**). Compound **1** it was isolated from a natural source for the first time.

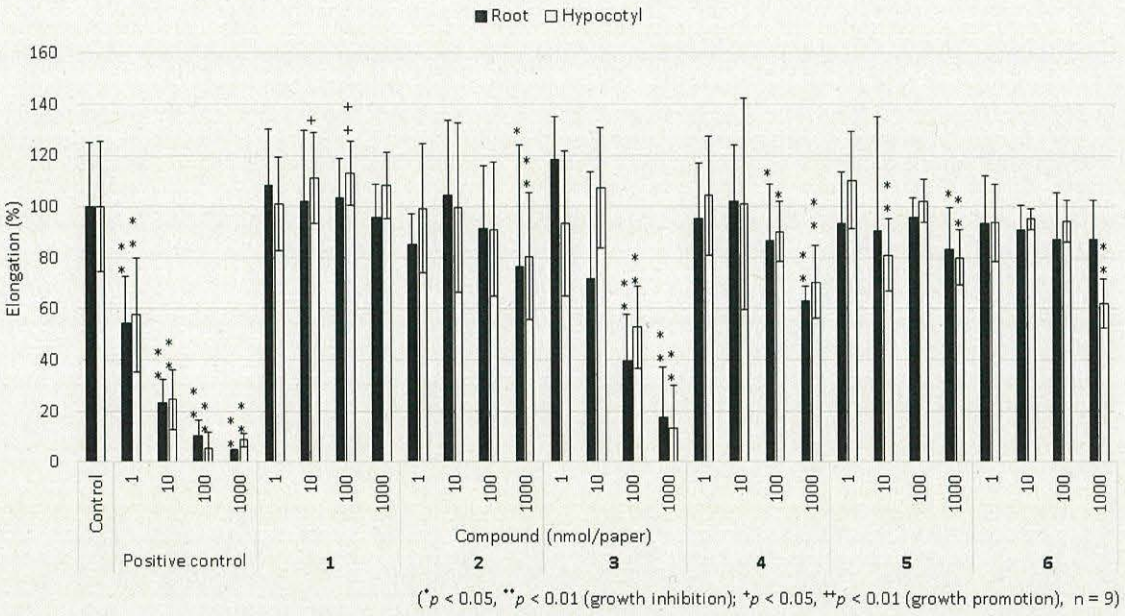
Furthermore, the absolute configuration of a malic acid derivative (**3**) that was previously isolated from the mushroom was determined. In this study, we synthesized stereoisomer of **3** (**4**) to determine the absolute configuration and also other two analogs (**5** and **6**) to study the structure activity relationship. As a result, compound **3** was determined to be *R*, indicating that it was a novel compound.



All the obtained compounds were evaluated their activities against the Axl and immune checkpoint assays and plant growth regulating activity assay. In the first assay, compounds **1** and **2** inhibited Axl, PD-L1, and PD-L2 expressions. Among malic-acid esters (**3**–**6**), only the isolated compound **3** showed the suppression effect on all the gene expressions.



In the second assay, plant growth regulating activity of **1**–**6** was evaluated using lettuce. As a result, compound **1** showed the promotion effect at 10 and 100 nmol/paper on hypocotyl growth, while **2** showing inhibition activity at 1000 nmol/paper against hypocotyl and root growth. The inhibition activity of the novel compound **3** was the strongest among all the compounds tested. The antipode of **3** (**4**) showed much less activity than **3**.

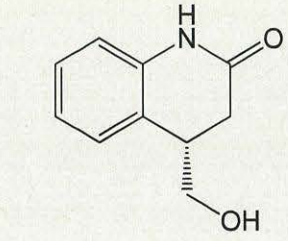


2) Bioorganic chemical studies on culture broth of *Stropharia rugosoannulata*

Stropharia rugosoannulata is a wild edible mushroom which commonly called Saketsubatake in Japanese or wine-cap stropharia in English, and it belongs to the *Strophariaceae* family. As an edible mushroom, *S. rugosoannulata* has some functional-food constituents possessing some pharmaceutical properties. As a continuous study, the chemical exploration of the culture broth of *S. rugosoannulata* was conducted along with the evaluation of the antibacterial activity and plant growth regulating activity.

S. rugosoannulata mycelia were cultured for 30 days. Afterwards, the mycelia were separated by filtration to obtain the culture broth, then concentrated under reduced pressure. The concentrated filtrate was partitioned into *n*-hexane, EtOAc, EtOH, and H_2O soluble parts. The EtOAc soluble part was subjected to silica gel flash column chromatography, which was further purified by HPLC. Structural determination of each compounds was performed by interpretation of IR, MS and NMR data. As a result, nine

compounds were isolated from the culture broth of *S. rugosoannulata* and identified as (*S*)-4-(hydroxymethyl)-3,4- dihydroquinolin-2(1H)-one (**7**), acetyl-*D*-phenylalanine (**8**), quinoline-4-carboxaldehyde (**9**), quinoline-4-carboxylic acid (**10**), (*R*)-1-phenylethane-1,2-diol (**11**), (1*R*,2*S*)-1-phenylpropane-1,2- diol (**12**), (*R*)-5-((*S*)-1-hydroxyethyl)dihydrofuran-2(3H)-one (**13**), 4-(2-hydroxyethyl)phenol (**14**), and 3,5-dichloro-4-methoxybenzoic acid (**15**). Compounds **7**, **8**, and **11** were isolated from nature for the first time.



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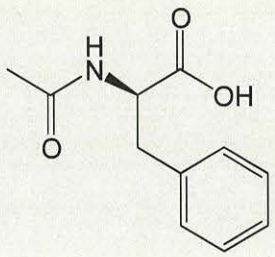

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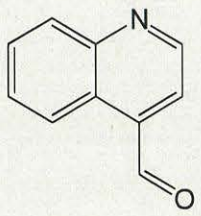

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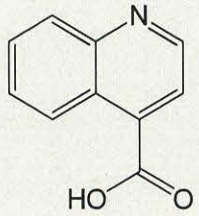

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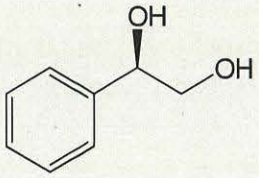

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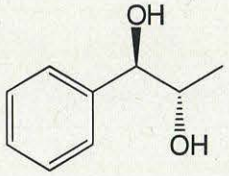

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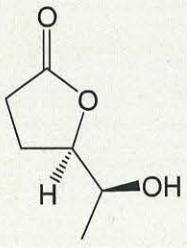

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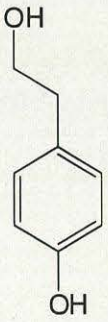

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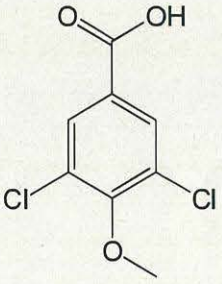

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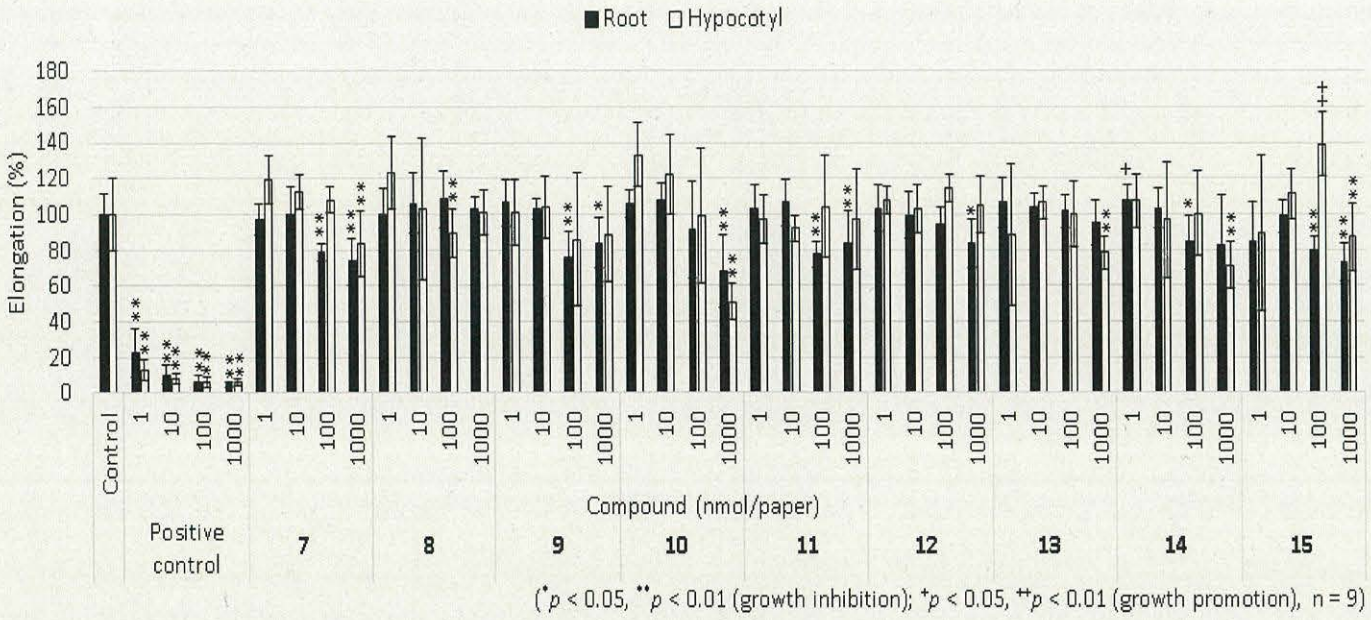
Antibacterial activity of compounds **7 – 15** was evaluated. As a result, all the compounds showed no antibacterial activity against *Clavibacter michiganensis, Pectobacterium carotovorum,* and *Burkholderia glumae.*

Plant growth regulating activity of **7–15** was evaluated using lettuce. all the compounds exhibited activity towards lettuce growth. However, compound **10** showed

the strongest inhibition towards lettuce growth on the plant growth regulation assay. According to the structure activity relationship study, the strong inhibition activity of **10** indicated that the presence of carboxylic acid group has an important role to suppress the growth of lettuce.

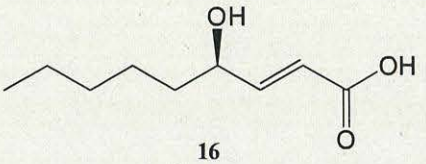


3) Bioorganic chemical studies on wild edible mushroom *Entoloma clypeatum*

Entoloma clypeatum is known as Harushimeji in Japanese and belongs to *Entolomataceae* family. This wild edible mushroom has been found as a good source of proteins and total carbohydrates with low fat contents. However, there is no report about isolation of bioactive compounds from the fungus. In this study, the chemical exploration of the fresh fruiting bodies of *E. clypeatum* was carried out.

Fresh fruiting bodies of *E. clypeatum* were extracted with EtOH and then acetone to obtain the crude extracts. The partition method divided them into n-hexane, EtOAc, and EtOH soluble parts. The EtOAc soluble part was subjected to silica gel flash column

chromatography, which was further purified by HPLC. Structural determination of each compounds was performed by interpretation of MS and NMR data. As a result, six known compounds were isolated and identified as (*R*,*E*)-4-hydroxynon-2-enoic acid (**16**), 9-methoxy-9-oxononanoic acid (**17**) 4-oxononanoic acid (**18**), methyl benzoate (**19**), Cyclo(*D*-Pro-*L*-val) (**20**), and nicotinic acid (**21**). Compounds **16** and **17** were isolated from mushroom for the first time.



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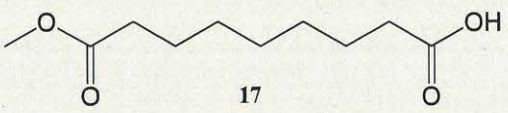

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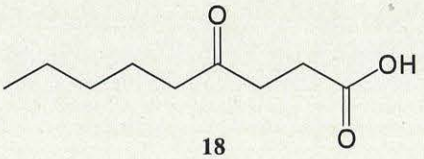

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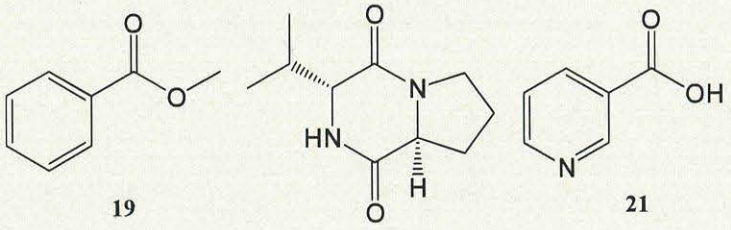

19 **21**

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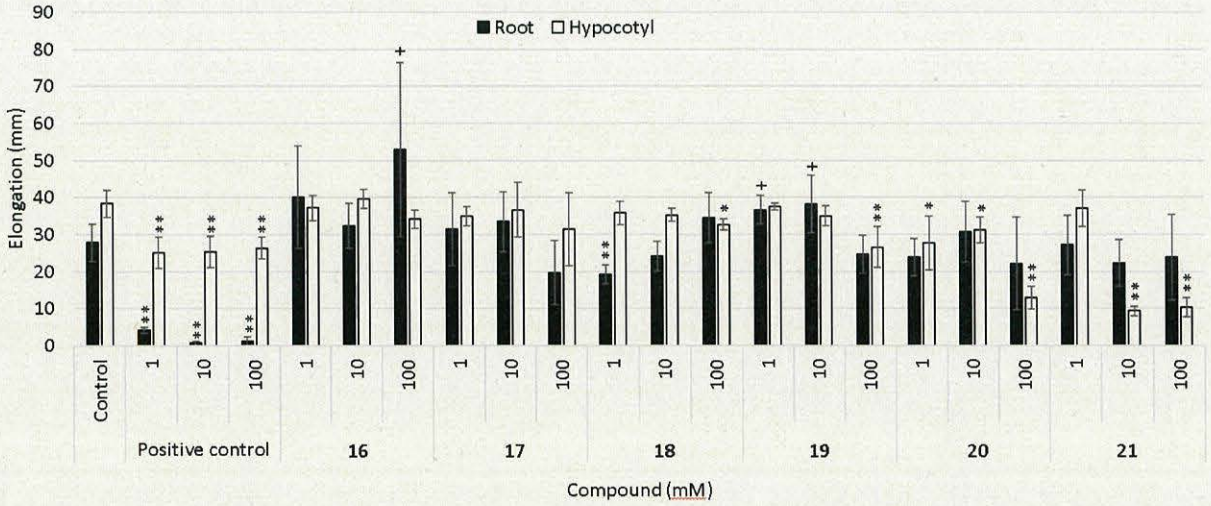
In the plant growth regulating activity assay, activity of **16–21** was evaluated towards rice seedlings. compound **16** showed strong promotion activity towards root growth, as well as compound **21** strongly inhibited hypocotyl growth.



($^{*}p < 0.05$, $^{**}p < 0.01$ vs control, $^{+}p < 0.05$, (growth promotion), n = 5)