Bioorganic Chemical Studies on Wild Edible Mushrooms

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：
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論文要旨：
Abstract：

Wild edible mushrooms are widely accepted as food and generally consumed worldwide as medicinal products. 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. 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*

Evaluation of chemical constituents and nutritional values of *Leucopaxillus giganteus* have been considerably studied. However, research about bioactive compounds from the mushroom is scanty, and only clitocine has been examined the biological activities, such as antitumor effect. In this study, the chemical exploration of mushrooms *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 comparison of MS and NMR data to the reported data. As a result, two known compounds were isolated and identified as ethyl 2-(2-oxopyrrolidin-1-yl)acetate (**1**) and monoethyl succinate (**2**).

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 assay. In the first assay, compounds **1** and **2** inhibited all the three genes expressions, Axl, PD-L1, and PD-L2. Among malic-acid esters (**3**–**6**), only the isolated compound **3** showed the suppression effects on all the gene expressions. In the

second assay, compound **1** showed the promotion effect at 10 and 100 nmol/paper against 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*

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, a novel compound, (*S*)-4-(hydroxymethyl)-3,4-dihydroquinolin-2(1H)-one (**7**), along with eight known compounds, 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**), were isolated from the culture broth of *S. rugosoannulata*. All the compounds showed no antibacterial activity against *Clavibacter michiganensis, Pectobacterium carotovorum,* and *Burkholderia glumae.* However, compound **10** showed the strongest inhibition towards lettuce growth on the plant growth regulation assay.

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

E. clypeatum is known as Harushimeji in Japan 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 along with the plant growth regulating assay.

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**). Compound **16** showed strong promotion activity towards root growth, as well as compound **21** strongly inhibited hypocotyl growth.