論文の内容の要旨

Search for anti-microbial compounds from cultured mycelia of *Basidiomycota* and *Ascomycota*

（担子菌門と子嚢菌門培養菌糸体の抗菌化合物の探索）

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Several kinds of mushrooms are well-known in traditional medicine in Asia. Recent studies showed they have various pharmaceutical activities such as food and drug intoxication, anti-hypertension and anti-cancer. Among them, *Basidiomycota* and *Ascomycota* species attract much attention because of their specialized secondary metabolites and pharmaceutical activities, for example, anti-tumor drugs Lentinan from *Lentinula edodes* and Schizophyllan from *Schizophylum commune*.

On the other hand, fungal infection happens often all over the world regardless species, gender or age. Nevertheless, antibiotic, salicylic acid and fatty acid type anti-fungal medicines were eliminated due to several reasons such as resistance, efficiency, side effects and the ability to making profits. And the tendency of anti-fungal drug elimination will accelerate in the future. Therefore, it is important to develop and discover new anti-fungal compounds/drugs before these problems become severe.

In this study, my aim was tried to achieve anti-fungal compounds. By using *Basidiomycota* and *Ascomycota* cultured mycelia of mushrooms to isolate anti-microbial compounds for treating skin or oral infection. Moreover, I also tried to uncover the secondary metabolites produced by these cultured mycelia.

Results

By means of paper-disk method, mycelium and culture broth from several kinds of *Basidiomycota*, and *Ascomycota* fungi were screened for the anti-microbial activity toward *Microsporum canis, Candida albicans* and *Trichophyton rubrum.* Among these fungi, *Lignosus rhinoceros, Isaria* sp. and *Ganoderma mastporum* showed strong bioactivities.

1-1 Anti-fungal compounds from *Lignosus rhinoceros*

L. rhinoceros were cultured in liquid potato dextrose (PD) medium, at 25℃ in the dark. Culture broth (20L) of *L. rhinoceros* were condensed by evaporator to 2L, and then extracted with ethyl acetate (EtOAc) and then *n*-butanol (BuOH). The EtOAc and BuOH extracts were tested for the bioactivities toward *M. canis*, *C. albicans and T. rubrum* as bioactivity-guide. Through the bioactivity-guided fractionation procedure, the bioactive compounds **1** and **2** were isolated by silica-gel column chromatography and NP- HPLC. Compounds **1** and **2** (Figure 1) were found to have a peak by LR-EI-MS at *m/z* 166 and 200, respectively. Together with ^{1}H-NMR, ^{13}C-NMR and HMBC, compound **1** was identified as 3,5-dimethoxybenzaldehyde, and compound **2** was identified as 2-chloro-3,5-dimethoxybenzaldehyde. This is the first report of anti-fungal compounds from *L. rhinoceros*.

1-2 Secondary metabolites from *L. rhinoceros*

Excepted the 2 bioactive compounds, 6 compounds (Figure 1) from the same fraction were isolated by silica-gel column chromatography and RP-HPLC named *rel*-(1*S*, 2*R*)-1-(3',5'-dimethoxyphenyl)propane-1,2-diol (**3**), *rel*-(1*R*, 2*R*)-1-(3',5'-dimethoxyphenyl)propane-1,2-diol (**4**), 3,5-dimethoxybenzoic acid (**5**), 2-chloro-3,5- dimethoxybenzoic acid (**6**), 6-(2-hydroxypropyl)-4-methoxy-pyran-2-one (**7**) and 1-(2-chloro-3,5-dimethoxy-phenyl)-ethane-1,2-diol (**8**).

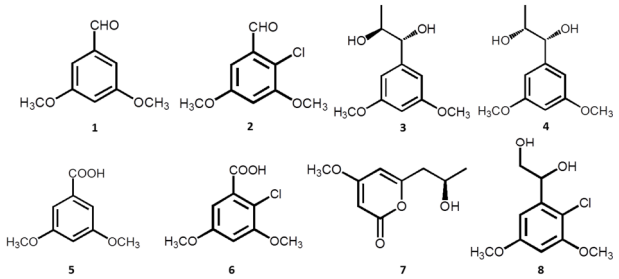

Figure 1. Structure of compounds **1-8**

Compounds **2**, **3**, **4**, and **7** are the first report from the natural product. Compound **8** is a new compound.

2-1 Anti-fungal compounds from *Isaria sp.*

The culture broth of *Isaria sp*. showed strong bioactivity toward both *M. canis* and *C. albicans*. As the result, large scale culture was carried out for compounds isolation. However, the bioactive fraction was seemed to exist as a compound which contains several conformations and is difficult to analyze by NMR. Several methods were tried in order to understand the structure including isolation properties,

methylation and acid treating. Unfortunately, larger amount of the sample is necessary for the further application.

2-2 Secondary metabolites from *Isaria sp*.

The mycelia of cultured *Isaria* sp. was freeze dried and then extracted with methanol for studying of its secondary metabolites. The extract was applied to silica-gel column chromatography, NP/RP-HPLC to give compounds **9**, **10**, **11**, **12** and **13** (Figure 2). According to NMR and MS spectra, compound **9**, **10, 11** and **12** are 3β,5α-dihydroxyergosta-7,22-dien-6-one, 5α,6α-epoxyergosta-7,22-dien-3β-ol, 3β,5α,9α-trihydroxyergosta- 7,22-dien-6-one and 6α,9α-epoxyergosta-7,22-dien-3β-ol, respectively. And compound **13** was identified as a new compound which was found to have a peak by HR-ESI-MS of *m/z* 467.3047 $[M+Na]^+$ with a molecular formula $C_{28}H_{44}O_4$.

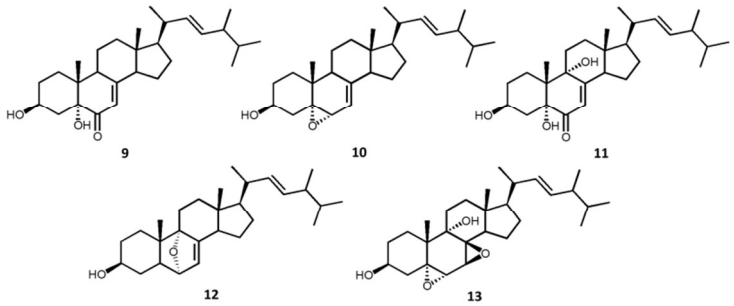

Figure 2. Structure of compounds **9-13**

3 Anti-microbial compounds from *Ganoderma mastporum*

According to paper disk method results of *M. canis*, mycelia extract of *G. mastporum* showed bioactivity. By means of bioactive guide fractionation method, the extract was applied to silica-gel column chromatography, NP/RP-HPLC to obtain compounds **14**, **15**, **16**, **17** and **18** (Figure 3). They are benzoic acid, *meso*-hydrobenzoin, ergosterol, ergosterol-peroxide and ergostatrien-3β-ol, respectively. The anti-fungal results showed compounds **14** and **17** have anti-fungal activity.

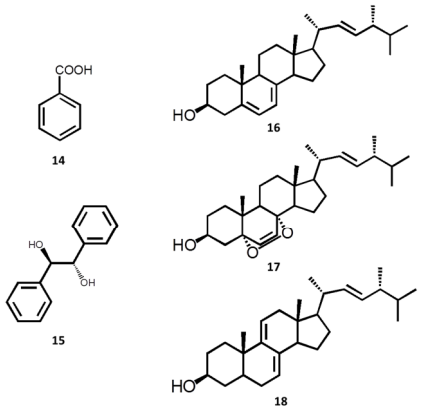

Figure 3. Structure of compounds **14-18**

Conclusion

By means of bioactivity-guided fractionation procedure, bioactive compounds **1** and **2** were isolated from *L. rhinoceros,* and compounds **14** and **17** were isolated from *G. mastoporum.* As to secondary metabolites discovery, 2 new compounds were isolated from this study. Nowadays, resistance to antibiotics in pathogenic fungi is a problem. And limited number of antifungal drugs makes this phenotype and acute problem. Investigation in this study will can give a help to solve this problem.