（第３号様式）(Form No. 3)

学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Isolation and Characterization of α-Glucosidase Inhibitor and Antioxidant Compounds from *Aspergillus terreus*

Aspergillus terreus からのα-グルコシダーゼ阻害物質および抗酸化物質の単離と特性評価

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Diabetes mellitus is now becoming a common metabolic disorder resulting from the inability of our body's response to high blood glucose level. Type 2 diabetes mellitus is reported to be 90-95% of all diabetic cases, characterized by hyperglycemia. There is considerable evidence that hyperglycemia results in the generation of reactive oxygen species, ultimately leading increased oxidative stress in a variety of tissues. Inhibition of α-glucosidase is one approach to suppress postprandial hyperglycemia, thereby delaying glucose absorption and controlling the hyperglycemia in diabetic patients and reducing chronic vascular complications. Therefore, the ideal antidiabetic compound should possess both hypoglycemic and antioxidant properties.

Aspergillus terreus is a filamentous, cosmopolitan and ubiquitous fungus which is primarily isolated from soil. The compounds isolated from *A. terreus* mostly possess pharmacological and commercial values, such as lovastatin as anti-cholesterol. However, there are only a few reports on production of α-glucosidase inhibitor and antioxidant by species of *Aspergillus*. Therefore, the aims of the study are to isolate and characterize the active compounds of *A. terreus* guided by inhibitory on yeast α-glucosidase and antioxidant assays. In this study we selected four isolates from *A. terreus* namely LS01 and RCC1 which cultured on potato malt peptone (PMP) medium, and MC751and LS07 which cultured on Czapek-dox (Cz) medium.

The isolation of antioxidant compounds from ethyl acetate (EtOAc) extract of LS01 using column chromatography on silica gel was guided by antioxidant activity yielded the crystalline compounds **1** and **2** which were identified as terreic acid and terremutin, respectively, on the basis of spectrometric analyses. Terreic acid (**1**) and terremutin (**2**) exhibited the highest level of DPPH free radical scavenging activity with

IC_{50} values of 115.0 and 114.0 μM, respectively. This is the first report on antioxidative activity of terreic acid (**1**) and terremutin (**2**) from *A. terreus*.

The bioassay-guided isolation and purification of an EtOAc extract of *A. terreus* MC751 led to the characterization of butyrolactone I (**3**) as an antidiabetic and antioxidant. The compound **3** demonstrated significant concentration-dependent, mixed-type inhibitory activity against yeast α-glucosidase with an IC_{50} of 52.2 μM. The antioxidative activity of compound **3** was evaluated based on the scavenging effects on DPPH with IC_{50} value of 51.4 μM. This is the first report on α-glucosidase inhibitory activity of butyrolactone I (**3**). Further studies revealed that the production of compound **3** and activities of EtOAc extract of MC751 increased when Czapek-dox medium was added with 0.5% yeast extract (CzY) and incubated under static condition for 15 days. The EtOAc extract was successively subjected to silica gel column chromatography to afford butyrolactone I (**3**) and II (**4**) as major constituents. In order to verify this structure and to study the structure activity relationship (SAR), compound **3** was converted to aspernolides A (**3a**) and acetylated to give butyrolactone I- triacetate (**3b**) and -diacetate (**3c**). The SAR of butyrolactone derivatives revealed that the prenyl side chain and hydroxyl group at lactone group in butyrolactone I has contribution for inhibitory α-glucosidase activity, however not for antioxidant activity. The compound **3** was found to be the most active compound as inhibitor of α-glucosidase (IC_{50}= 52.2 μM), while compound **4** was the most potential for antioxidant (IC_{50}= 17.6 μM).

A. terreus LS07 was cultured in CzY medium under static condition for fifteen days (i) and shaking condition for seven days (ii) in order to increase the yield of extract. Chromatographic separation of the EtOAc extract (i) led to the isolated of compounds **3** and **4.** The isolation of the EtOAc extract (ii) gave compound oleic acid (**5**), ergosterol (**6**) beside compounds **3** and **4** as major metabolites. Ergosterol (**6**) exhibited poor inhibitory activities on α-glucosidase and scavenging DPPH radicals. In particular oleic acid (**5**) showed the highest α-glucosidase inhibitory activity (IC_{50}=8.5 μM). By comparing the activity of stearic, linoleic, and linolenic acid, it revealed that a double bond in compound **5** was essential for α-glucosidase inhibition. However, the increasing of the double bond number in unsaturated fatty acid will decrease the α-glucosidase inhibitory activity.

Five compounds were isolated from *A. terreus* RCC1 and their structures were identified as butyrolactone I (**3**), lovastatin (**7**), terrein (**8**), isoaspulvinone E (**9**), and aspulvinone E (**10**). Isoaspulvinone E (**9**) and aspulvinone E (**10**) exhibited high activity on α-glucosidase inhibitory with IC_{50} value of 8.9 and 2.7 μM, respectively. Moreover, these compounds exhibited the highest level of DPPH free radical scavenging activity with IC_{50} values of 167.8 and 114.8 μM, respectively. Up to our knowledge, this is the first report of inhibitory activity on α-glucosidase and antioxidant of aspulvinone compounds. The results of this study revealed that *A. terreus* could be considered as a potential source of natural antioxidant and antidiabetic drug candidates.