

(別紙様式 6)  
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学位論文の要旨 (論文の内容の要旨)  
Summary of the Dissertation (Summary of Dissertation Contents)

論 文 題 目

Medicinal and Pharmaceutical Properties of Momilactones A and B

広島大学大学院国際協力研究科  
Graduate School for International Development and Cooperation,  
Hiroshima University  
博士課程後期 開発科学専攻  
Doctoral Program Division of Development Science  
学生番号 D161608  
Student ID No.  
氏 名 □  
Name NGUYEN VAN QUAN Seal

**Background**

Momilactones belong to diterpenes group which are typically characterized by a lactone ring linked with the pimarane skeleton. According to the first definition of “momilactone” described by Kato and colleagues in 1973, every lactone structure derived from the rice husk (“籾” - “momi”) can be named as a momilactone. Among identified momilactones, momilactones A (MA) and B (MB) are the most common but only detected in rice (*Oryza sativa*) and the moss *Hypnum plumaeforme*. Previous studies soon manifested the potent allelopathic function of MA and MB against plant’s natural enemies such as weeds and blast fungus. Later studies confirmed these compounds are authoritative allelochemicals against several pathogens of crops as well. Recently, MA and MB were found more implicative with salinity and drought tolerance of rice more than allelopathy. They are also exhibited antioxidant, cytotoxic, antitumor and anticancer activities. Though MA and MB are promising bioactive constituents in rice, the isolation and purification of the metabolites are complicated and laborious. At present, there are very few laboratories in the world that can successfully isolate and purify MA and MB. As a result, no commercial MA and MB from chemical companies in Japan or abroad can be purchased; thus, knowledge on medicinal and pharmaceutical properties of these compounds has been limited.

**Main objectives**

Therefore, this research was conducted to at first examine medicinal and pharmaceutical properties of pure momilactones A and B isolated from rice husks focusing on anti-diabetes, anti-obesity, antioxidant and anti-skin-aging; and, to determine the potential sources of momilactones A and B by quantifying their contents among rice plant parts (bran and endosperm) and among common rice varieties.

**Structure of dissertation**

- Chapter 1: Introduction
- Chapter 2: Isolation and purification of momilactones A and B and momilactone-like compounds from rice husk
- Chapter 3: Momilactones A and B are  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors
- Chapter 4: Contributions of momilactones A and B to diabetes and obesity inhibitory potentials of rice bran: Evidence from in vitro assays
- Chapter 5: Antioxidant and anti-skin-aging properties of momilactones A and B
- Chapter 6: General discussion and conclusion

**Materials and methods**

**Materials:** Rice husk of *Oryza sativa* (var. Koshihikari) was collected in Higashi-Hiroshima. Chemicals and reagents at high grade were purchased from prestigious providers including Junsei Chemical Co., Ltd., Fujifilm Wako Pure Chemical Corporation, Japan, Fisher Scientific company, and Sigma-Aldrich, USA.

**Methods:**

- Pure compounds were isolated and purified by repeated normal phase column chromatography over silica gel;
- Momilactones were identified and confirmed by reliable advance-spectroscopic techniques consisting of high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), gas chromatography-

mass spectrometry (GC-MS), liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS),  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR);

- Biological activities were assayed *in vitro* by using antioxidant and inhibitory experiments on targeted enzymes relevant to diabetes, obesity and skin-aging comprising  $\alpha$ -amylase,  $\alpha$ -glucosidase, pancreatic  $\alpha$ -amylase, trypsin, elastase, and tyrosinase.

### Main results

In chapter 2, four bioactive compounds including momilactone E (ME), 7-ketostigmaterol (7KS), momilactone A (MA), and momilactone B (MB) were isolated by column chromatography. The inhibitory activities of MA, MB, ME, and 7KS were examined on lettuce (*Lactuca sativa*), barnyard grass (*Echinochloa crus-galli*), and tall goldenrod (*Solidago altissima*) in bioassays. The allelopathic activities of ME and 7KS were compared with those of potent phytoalexin momilactones A (MA) and B (MB), and the standard *p*-hydroxybenzoic acid (*p*HA). Results showed that both MA and MB exhibited stronger inhibitory activity than ME and 7KS. MB exerted greater inhibitions than MA but the mixture of MA and MB (1:1, *v/v*) possessed a similar level of inhibition to MB. On the other hand, although ME and 7KS presented non-significant inhibition, their mixture of ME-7KS (1:1, *v/v*) displayed a remarkable inhibition on the growth of *S. altissima*. Findings of this chapter revealed that MA, MB, and the mixture ME-7KS had the potential to control the invasive plant *S. altissima* and the noxious paddy weed *E. crus-galli* *in vitro*, but their mode of actions should be further investigated.

In chapter 3, the results of enzymatic *in vitro* assays showed that both MA and MB exerted potent inhibition on  $\alpha$ -amylase and  $\alpha$ -glucosidase activities. The inhibitory effect of MB on these two key enzymes was greater than that of MA. Both MA and MB exerted greater  $\alpha$ -glucosidase suppression as compared to that of the commercial diabetic inhibitor acarbose. Quantities of MA and MB in rice grain were 2.07 and 1.06  $\mu\text{g}/\text{dry weight}$  (DW), respectively. This study was the first to confirm the presence of MA and MB in refined rice grain and reported the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity of the two compounds.

Chapter 4 was the first to detect the presence of the two compounds MA and MB in rice bran using the LC-ESI-MS technique. By *in vitro* assays, both MA and MB exhibited potent inhibitory activities on pancreatic  $\alpha$ -amylase and  $\alpha$ -glucosidase which were significantly higher than  $\gamma$ -oryzanol, a well-known diabetes inhibitor in rice bran. Remarkably, MA and MB indicated an effective inhibition on trypsin with the  $\text{IC}_{50}$  values of 921.55 and 884.03  $\mu\text{g}/\text{mL}$ , respectively. By HPLC, quantities of MA (6.65  $\mu\text{g}/\text{g}$  dry weight) and MB (6.24  $\mu\text{g}/\text{g}$  dry weight) in rice bran were determined. Findings of this study revealed the  $\alpha$ -amylase,  $\alpha$ -glucosidase and trypsin inhibitors MA and MB contributed an active role to the diabetes and obesity inhibitory potentials of rice bran.

Chapter 5 reported the potential antioxidant and anti-skin-aging activities of MA and MB. Results from antioxidant assays presented a synergistic antioxidant activity of the MA and MB (MAB, 1:1, *v/v*) by ABTS and reducing power assays. Remarkably, in ABTS assay,  $\text{IC}_{50}$  value of MAB (0.319  $\text{mg}/\text{mL}$ ) was 4 folds and 9 folds greater than that of individual MB (1.28  $\text{mg}/\text{mL}$ ) or MA (2.84  $\text{mg}/\text{mL}$ ), respectively. Enzymatic assays on pancreatic elastase and tyrosinase indicated that MA and MB were potential cosmeceuticals in protecting the skin from wrinkles and freckles. Besides, the validated method for quantification of MA and MB in rice grains showed that brown rice grains contained more MA and MB than white rice grains due to the MA and MB involvement in bran. Among refined grains, Koshihikari possessed the highest amount of these active compounds. The usage of a specific technique in preparation of samples and an advanced UPLC-ESI-MS method helped improve the sensitivity of the quantification of MA and MB in various rice grains.

### Conclusion

This study showed the first-time inhibitory effects of momilactones A (MA) and B (MB) on the key enzymes related to human diseases including diabetes, obesity, and skin aging. The results can be applied widely in medicine and contribute to the development of the prospective therapeutics. Significantly, for the first time, the present research successfully detected and quantified MA and MB in bran and white rice grains. Since most of the studies agree with the fact that white rice intake can increase diabetes risk, the existence as diabetic and obesity inhibitors of MA and MB in white rice grain may alter the antecedent perspective that white rice consumption induced risks of type 2 diabetes and obesity. The HPLC/UPLC-ESI-MS methods and the protocol of sample preparation were validated, which helped determine and quantify MA and MB at higher reliability and sensitivity. However, further studies should be implemented to assert applicable doses of MA and MB before conducting medicinal production, pre-clinical and clinical trials on the two compounds. Finally, given that quantities of MA and MB are largely varied among rice cultivars, the breeding of new rice cultivars with high amounts of MA and MB may be useful and economical to help control several chronic diseases in human.

備考 論文の要旨はA4判用紙を使用し、4,000字以内とする。ただし、英文の場合は1,500語以内とする。

Remark: The summary of the dissertation should be written on A4-size pages and should not exceed 4,000 Japanese characters. When written in English, it should not exceed 1,500 words.