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Quantification of Momilactones A and B in Rice Straw

Choon-Woo Lee*†, Koichi Yoneyama, Yasutomo Takeuchi**, and Su Noh Ryu*****

*National Crop Experiment Station, RDA, Suwon 441-857, Korea
**Center for Research on Wild Plants, Utsunomiya University 350 Mine-machi, Utsunomiya, Tochigi 321-8505, Japan
***Dept. of Agromy, Korea National Open University, Seoul 110-791, Korea

ABSTRACTS : Momilactones A and B, the major phytotoxins and phytoalexins in rice plants, were quantified by a HPLC-APCI-MS-MS (APCI-MS-MS) system under multiple reaction monitoring conditions. Since MA and MB were found to be easily extracted with water, these phytotoxic compounds may affect germination and growth of other plant species when the rice straws were left in the fields.

Keywords : Momilactone A, Momilactone B, HPLC-APCI-MS-MS system.

Rice straws are usually left in the paddy fields after harvest. Allelochemicals from the straws due to soil moisture, rain and microorganisms are released into the soil by leaching and decomposition.

There has been a number of reports about allelopathic effect of rice plants (*Oryza sativa* L.). The inhibitory activity of different varieties, extraction methods (Lee *et al.*, 1998), and the contents or composition of allelochemicals in rice is diverse. Allelopathic compounds in rice straw were identified mainly as phenolic compounds (Olofsdotter *et al.*, 1995). Since Momilactone A and B, and oryzalexines A and C from rice husk as major growth inhibitors against several weed species (Kato *et al.*, 1973).

In a preliminary experiment, phytotoxic compounds in rice straws were examined. Momilactone A (MA) was identified as major growth inhibitor against several weed species tested. They have been shown to participate in plant defense system against pathogens (Cartwright, 1981). Phenolic compounds had weak inhibitory activities and were utilized as nutrient sources or converted into non-toxic forms by microorganisms in the soil. Several researches isolated momilactone A and found that the endogenous levels of Momilactone A and B are increased by biotic and also by abiotic stresses. A simple and reliable quantification method for MA is therefore needed to clarify the allelopathic potential of rice straws left in the fields.

†Corresponding author: (Phone) +82-31-290-6675 (E-mail) leecw@rda.go.kr <Received July 23, 2002>

Quantification of authentic MA could be done by gas-liquid chromatography-mass spectrometry (GC-MS) without derivation, whereas MB could not be analyzed without being converted to TMS ether (Kawaguchi *et al.*, 1997). Therefore, qualification of MB by GC-MS requires an adequate purification process and subsequent derivation before analysis. These compounds could be quantified by the LC-MS-MS system without complicated purification and derivation processes and the LC-MS-MS system may afford a simple and useful quantification method for a wide range of natural products. This study describes a high-performance liquid chromatography-atmospheric chemical ionization tandem mass spectrometry (HPLC-APCI-MS-MS) system for the quantification of both MA and MB, and its application to the quantification of MA in rice straws.

MATERIALS AND METHODS

Momilactone A was obtained from methanol extract of dried rice straws by a conventional purification procedure as described in the literature (Lee *et al*, 1999a; Kato *et al*, 1977). Details of purification process was follow as lee's method (Lee *et al.*, 1999b). Their structures were confirmed by spectroscopic analyses (NMR and mass spectrometry).

High-performance liquid chromatography was done using HP 1100 HPLC Series (Hewlett-Packard, Waldbronn, Germany) equipped with an Inertsil ODS-2 column (150 mm × 4.6 I.D., 5 mm, GL Sciences, Japan). Elution with 80% aqueous acetonitrile (containing 0.1% formic acid) was carried out at a flow-rate of 0.6 ml/min.

Mass spectrometry was analyzed with a Sciex API-300 (Perkin-Elmer SCIEX Instruments, Foster City, CA, USA) equipped with an APCI inlet system for the HPLC-APCI-MS-MS system. All the tested compounds were analyzed in a positive-ion mode. Nitrogen was used as the collision gas. State files are as follows; NEB = 10, CUR = 10, CAD = 4, NC = 3, TEM = 425, OR = 25, RNG = 250, Q0 = -10, IQ1 = -11, ST = -11, RO1 = -12, IQ2 = -34, RO2 = -29, IQ3 = -54, DF = -100, CEM = 1900. Quad 1: 30 (0.066), 100 (0.11), 1000 (0.473), 2000 (0.875). Quad 3: 10 (0.025), 100

(0.055), 1000 (0.412), 2000 (0.750). The multiple reaction monitoring (MRM) mode was used to monitor the parent and product ions. The dwell time was set at 500 ms and the duration time was 10 min. The pause time was 5.0 ms.

Plant materials and samples were prepared as described in the literature (Lee *et al.*, 1999a).

RESULTS AND DISCUSSION

Ion spray MS-MS conditions for momilactones A and B

IS ionization and the subsequent fragmentation by N_2 gas give a set of the parent ion and its product ion(s) with MS-MS. If a particular set of parent and its product ions are properly selected, quantification of target molecule is possible with high accuracy and sensitivity by minimizing background noise. This method is called multiple reaction monitoring (MRM). The parent ion (M+1) of MA appeared at m/z 315, and its product ion appeared at m/z 271, probably due to the loss of lactone moiety (-44, COO), by MS-MS. In the case of MB, M + Na (m/z 353) ion was observed as well as the parent ion at m/z 331, and the parent ion of MB afforded two product ions at m/z 313 (-18, H_2O) and 269 (313-44), as minor and major ones, respectively (Fig. 1). Therefore, combinations of m/z 315/271 and 331/269 were selected for quantification of MA and MB.

Quantification of momilactones A and B in rice straws

Since MA and MB are hydrophobic, atmospheric pressure chemical ionization (APCI) was preferred. Standard and extracted samples were injected into the HPLC-APCI-MS-MS system under MRM analytical conditions. The detection limit of MA and MB is approximately between 0.5 to 1 ng. The retention times of the authentic compounds are 5.23 min for MA and 4.43 min for MB (Fig. 1) at a flow rate of 0.6ml/min. Calibration curves for MA and MB were obtained using two standards of these compounds (0.5 and 2.5 ng) just before the analyses of extracted samples. The analyte standard curves were calculated using the SCIEX MacQuan 1.5 program. Analyte ions in the samples were monitored by MRM, and the concentration of the analyte was determined. Fig. 2 shows the typical MRM spectra for MA of authentic and extracted samples, and demonstrates the effectiveness of this analytical method for quantification of MA and MB.

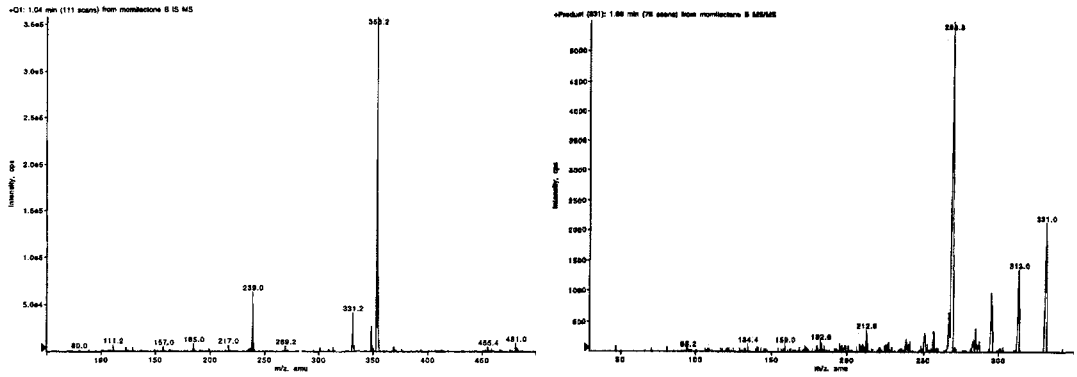

Fig. 1. Parent ion (left) and product ion (right) of momilactone B.

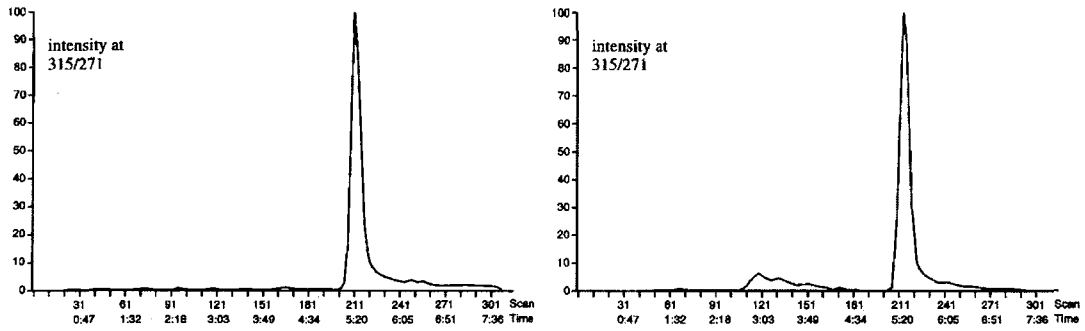

Fig. 2. Typical MRM spectra for MA of authentic (left) and extracted samples (right).

In the rice (cultivar : Haresugata), the concentrations of MA were always higher than those of MB (Lee *et al.*, 1999a). Approximately 30-40% of these phytotoxins were extracted with water. These results indicate that MA and MB in rice straws are easily extracted with water and that these phytotoxic compounds may cause some inhibitory effects on the germination and growth of susceptible plant species under field conditions.

Momilactone A (MA) identified as major growth inhibitor showed to participate in plant defense system against pathogens (Cartwright, 1981); otherwise phenolic compounds had weak inhibitory activities against microorganisms in the soil. Several researches isolated momilactone A and found that the endogenous levels of Momilactone A is increased by biotic and also by abiotic stresses. A simple and reliable quantification method for MA is therefore needed to clarify the allelopathic potential.

Quantification of authentic MA could be done by gas-liquid chromatography-mass spectrometry (GC-MS) without derivation, whereas MB could not be analyzed without being converted to TMS ether (Kawaguchi *et al.*, 1997). But even without these processes, MA and MB may still be quantified in this study.

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