# Biotransformation of Benzyl Benzoate from Benzoic Acid in Rice Watery Ovipositional Lesion Tissues Induced by *Sogatella furcifera* (HORVÁTH) (Homoptera: Delphacidae)[1]

Yoshito SEINO[2] and Yoshito SUZUKI

*Kyushu National Agricultural Experiment Station, Kumamoto 861–11, Japan*

(Received 21 February 1997)
(Accepted 14 April 1997)

*Key words*: benzoic acid, benzyl benzoate, *Sogatella furcifera*, biosynthesis

Benzyl benzoate (BB) is known as an acaricidal substance (SATO et al., 1989). Recently we found that BB is produced by rice plants and shows ovicidal activity *in vitro* against the whitebacked planthopper, *Sogatella furcifera* HORVÁTH (SEINO et al., 1996). BB production in rice plants is specific to watery ovipositional lesion tissues and has not been confirmed in non-watery ovipositional lesions or intact plant tissues. More than 80% of *S. furcifera* eggs die at early embryonic development stages in watery ovipositional lesions, while 88.8% of eggs develop to the eyespot formation stage in non-watery ovipositional lesions (SUZUKI et al., 1996).

BB is found in some plants (OGUNTIMEIN et al., 1989; NKUNYA et al., 1990; JANTAN, 1990; HISHAM et al., 1991). CROTEAU (1977) experimentally demonstrated the pathway for biosynthesis of BB in ripe cranberry. Using $^{14}C$-labeled substances, he proposed the following pathway: Benzoic acid is reduced to benzyl alcohol (BO) via benzaldehyde (BA) and then benzoyl-coenzyme A derivative is combined with BO to produce BB. ISHII et al. (1962) has shown that rice plants contain benzoic acid which shows growth-inhibiting activity against larvae of the rice stem borer, *Chilo supressalis* WALKER. In addition, BO and BA have been detected in rice bran (TSUGITA et al., 1978). These findings suggest the possibility that the biosynthesis of BB in watery ovipositional lesion tissues in rice plants is based on the same pathway as that proposed for ripe cranberry by CROTEAU (1977). We tested this possibility using $^{13}C_6$-ring-benzoic acid which was injected into watery ovipositional lesions in rice plants.

## MATERIALS AND METHODS

*Plants.* Seedlings of a japonica rice variety, Reiho, were individually transplanted in 220 ml plastic cups after 3-d germination at 25°C, and cultivated in an outdoor growth cabinet controlled at 14L24°C: 10D20°C. Fertilizer was applied once 4 weeks after transplanting. Plants at the maximum tillering stage were used for the experiments after the small tillers were removed because the ovicidal reaction against *S. furcifera* is weak on small tillers (SUZUKI et al., 1996).

*Preparation of $^{13}C_6$-ring-benzoic acid water solution.* $^{13}C_6$-ring-benzoic acid purchased from Cambridge Isotope Laboratories ($^{13}C$, 99%) was dissolved in hot water and the solution was cooled to room temperature. It was adjusted to a concentration of 100 ppm by addition of water.

*Sampling plant tissues.* Gravid *S. furcifera* females were obtained from a stock colony maintained at the Kyushu National Agricultural Experiment Station on small Reiho seedlings since 1989. The insects were released to rice plants which were individually covered with a clear plastic cylinder with a tetron gauze cap, and allowed to oviposit for 1 d at 25°C. After the insects were removed, a water solution of $^{13}C_6$-ring-benzoic acid was injected into lesions with a fine glass capillary tube until it overflowed from the injection point. The injected plants were kept for 1 d prior to sampling of the plant tissues.

*GC-MS analysis of labeled substance from watery oviposition lesions.* Sample tissues were immersed in a tenfold volume of MeOH for 1 d at 25°C, and the extract was collected by filtration. The filtrate was dehydrated with anhydrous $Na_2SO_4$ and then MeOH was removed under reduced pressure. The extract was filled with 0.5 ml MeOH and analyzed by GC-MS under the following conditions: Instrument, JEOL JMS-600W mass detector equipped with Hewlett-Packard 5890 series II gas chromatograph; column, HP-5 fused silica column (Hewlett-Packard, 0.25 mm i.d.×30 m, 0.25 μm film thickness); column oven temperature program, 50°C for 1 min, 50 to 280°C at 10°C/min, and 280°C for 6 min; carrier gas, helium at a flow rate of 1 ml/min; mass detector,

[1] Appl. Entomol. Zool. **32** (3): 530–532 (1997)

[2] Present address: *Agricultural Chemicals Inspection Station, Suzuki-cho, Kodaira, Tokyo 187, Japan*

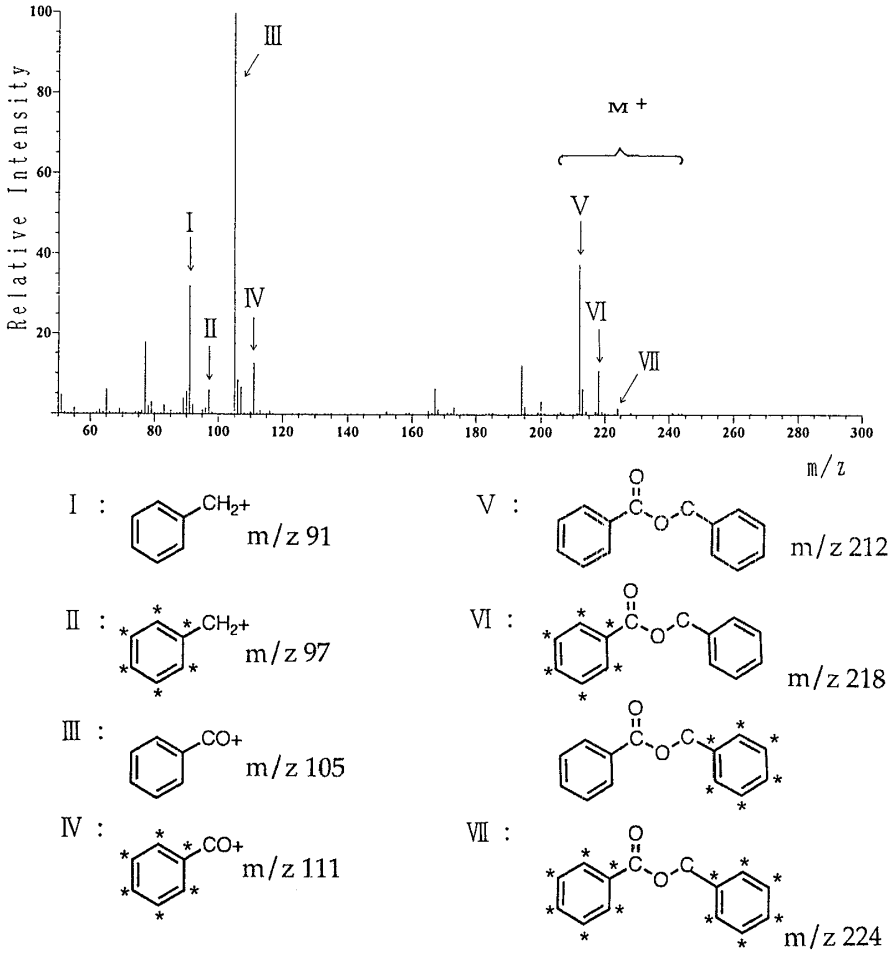


Fig. 1. Mass spectrum of benzyl benzoate in watery ovipositional lesion tissues which were injected with $^{13}C_6$-ring-benzoic acid. I, II, III and IV indicate characteristic fragment ion peaks and V, VI and VII indicate molecular ion peaks. The structures of fragment ions and molecules are shown below the mass spectrum. Asterisk (*) indicates the position of $^{13}C$.

EI mode at 70 eV. For comparison of mass spectrum, non-labeled BB purchased from Wako Pure Chemical Industries, Ltd. was analyzed under the same conditions.

After the analysis described above, the sample, to which trimethylsilyldiazomethane (Tokyo Chemical Industry Co., Ltd., 10% in *n*-hexane) was added for methylation of benzoic acid, was resubmitted for GC-MS analysis under the same conditions.

## RESULTS AND DISCUSSION

The results of the GC-MS analysis showed that the sample tissues (65.3 cm-long; 1.42 g) contained 15.6 ppm of BB. The mass spectrum of BB indicated 4 distinct molecular ions of BB (Fig. 1). The molecular ions of *m/z* 212 and 213 were observed in authentic BB (Fig. 2) and also in watery ovipositional lesion tissues which were not injected with $^{13}C_6$-ring-benzoic acid or authentic BB. The *m/z* 218 and 224 indicate that the molecular ions of BB contain single and double $^{13}C_6$-ring-benzene, respectively. The results showed that $^{13}C_6$-ring-benzoic acid was used for biosynthesis of BB in watery ovipositional lesion tissues. Further, the presence of BB constructed of double $^{13}C_6$-ring-benzene suggests that benzoyl coenzyme A derived from $^{13}C_6$-ring-benzoic acid was combined with benzyl alcohol derived from $^{13}C_6$-ring-benzoic acid. This suggestion was also supported by characteristic fragment ions at *m/z* 97 and 111 which correspond to $^{13}C_6H_5$-$CH_2^+$ and $^{13}C_6H_5$-$CO^+$, respectively. Thus the mass spectrum of BB provided evidence for the pathway for BB biosynthesis originally proposed

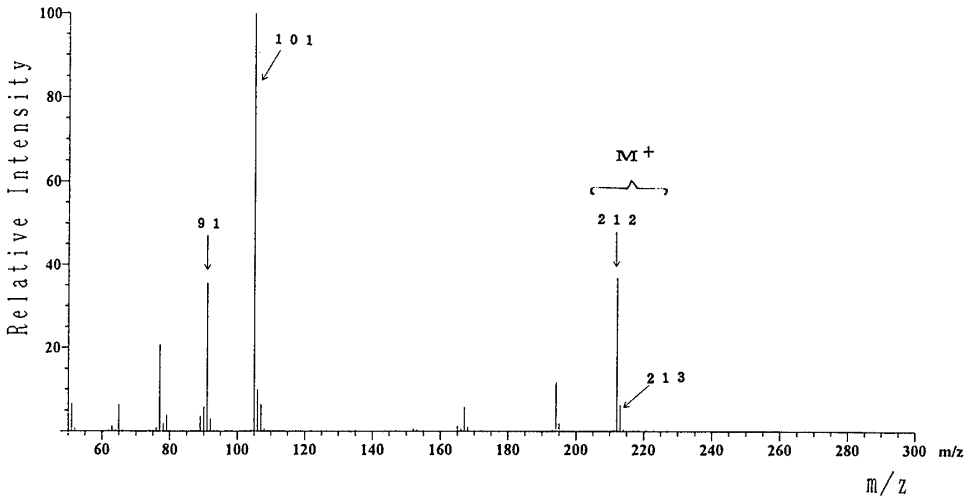


Fig. 2 Mass spectrum of authentic benzyl benzoate.

for ripe cranberry by CROTEAU (1977).

BA and BO, supposed intermediary metabolites in the pathway of BB, were not detected in the sample. Further study is needed to prove that BA and BO are intermediate products in the pathway. Benzoic acid as methyl ester contained in watery ovipositional lesion tissues was 19.5 ppm, and the ratio of non-labeled benzoic acid to $^{13}C_6$-ring-benzoic acid was about 4:1 according to the ion peaks at *m/z* 136 and 142. In addition, BB concentration in watery ovipositional lesion tissues was 15.6 ppm, and the ratio of non-labeled BB, single and double $^{13}C_6$-ring-benzene labeled BB was 52:11:2, according to the ion peaks at *m/z* 212, 218 and 224. These suggest that watery ovipositional lesion tissues synthesize BB continuously because the residue of benzoic acid as a precursor of BB was confirmed in the sample. Further study is needed to confirm these possibilities.

Benzoic acid is known as a phytoalexin of *Pinus radiata* against *Dothistroma pini* (FRANICH et al., 1986). The lesions of *P. radiata* needles induced by dothistromin, which is a *D. pini* toxin, synthesize and accumulate benzoic acid. Benzoic acid contained in rice plants also has an antifungal activity *in vitro* against the rice blast fungus, *Pricularia oryzae* and *Cochliobolus miyabeanus* (ISHII et al., 1962). These studies suggest that some plants synthesize benzoic acid and related substances for protection against a variety of pests.

## REFERENCES

CROTEAU, R. (1977) *J. Food Biochem.* **1**: 317–326.

FRANICH, R.A., M.J. CARSON and D. CARSON (1986) *Physiol. Molec. Plant Pathol.* **28**: 267–286.

HISHAM, A., L.A.C. PIETERS, M. CLAEYS, H. VAN DEN HEUVEL, E. ESMAN, R. DOMMISSE and A.J. VLIETINK (1991) *Phytochemistry* **30**: 2373–2378.

ISHII, S., C. HIRANO, Y. IWATA, M. NAKASAWA and H. MIYAGAWA (1962) *Jpn. J. Appl. Entomol. Zool.* **6**: 281–288.

JANTAN, I. (1990) *J. Tropical Forest Sci.* **2**: 252–259.

NKUNYA, M.H.H., H. ACHENBACH, C. RENNER, R. WAIBEL and H. WEENEN (1990) *Phytochemistry* **29**: 1261–1264.

OGUNTIMEIN, B., O. EKUNDAYO, I. LAKES and R. HILTUNEN (1989) *Planta Med.* **55**: 312–313.

SATO, H., H. KOBAYASHI, I. TSUKINAGA, M. ANDO and A. OHKAWARA (1989) *Hokkaido J. Med. Sci.* **64**: 139–145.

SEINO, Y., Y. SUZUKI and K. SOGAWA (1996) *Appl. Entomol. Zool.* **31**: 467–473.

SUZUKI, Y., K. SOGAWA and Y. SEINO (1996) *Appl. Entomol. Zool.* **31**: 111–118.

TSUGITA, T., T. KURATA and M. FUJIMAKI (1978) *Agric. Biol. Chem.* **42**: 643–651.