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学 位 論 文 要 旨

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題目: Natural Product Chemical Study on Biologically Active Secondary Metabolites Produced by Phytopathogenic Fungi.

（植物病原菌が生産する生理活性二次代謝産物に関する天然物化学的研究）

Fungal plant diseases are serious problems in agriculture all over the world. Phytopathogenic fungi produce diverse secondary metabolites that show phytotoxic and/or antimicrobial activity and that play important roles in their infection and colonization in plants. The accumulation of knowledge of the molecular infection mechanisms by plant pathogens would certainly contribute to the development of effective new and eco-friendly methods for protecting crop plants from many plant diseases. At the same time such molecules could afford lead structures for agrochemicals.

Radicinin is a phytotoxic and antibiotic metabolite produced by some phytopathogenic fungi. *Bipolaris coicis* H13-3 produces radicinin and its analogues, 3-*epi*-radicinin, 3-*epi*-radicinol and its epoxide. Their structures suggested a biosynthetic relationship between these metabolites; both radicinin and 3-*epi*-radicinin are synthesized from deoxyradicinin and 3-*epi*-radicinin is then reduced to 3-*epi*-radicinol, which is oxidized to 3-*epi*-radicinol epoxide. To confirm the conversion of deoxyradicinin to radicinin and 3-*epi*-radicinin, deoxyradicinin was administered to the fungus. The amount of radicinin detected was about eight times more than that of the control, and the amount of 3-*epi*-radicinin also increased as compared to the control. When radicinin was administered, there was about a 4-fold increase in the amount of 3-*epi*-radicinin compared with the control. These results indicated that deoxyradicinin is a direct precursor of radicinin and also that the fungus have an epimerizing enzyme which catalyzes the conversion of radicinin to 3-*epi*-radicinin. For further confirmation of the conversions, the experiment using a cell-free system prepared from *B. coicis* H13-3 was carried out. Incubation of deoxyradicinin with the cytosolic fraction from *B. coicis* gave rise to the enzymatic formation of radicinin. The activity of deoxyradicinin monooxygenase was measured at various conditions with the cytosolic fractions, indicating that the optimum temperature and pH for the enzyme activity was at 35 °C and pH 7.0, and that this enzyme preferred NAD^+ to other co-enzymes. The molecular weight of the monooxygenase was determined to be 130–184 kDa. Incubation of radicinin with the cytosolic fraction caused an increase in 3-*epi*-radicinin. The radicinin epimerase was purified, and its activity was measured at various conditions. The highest activity of the epimerase was found at 30–35 °C and pH 7.0–9.0,and the epimerase did not require any co-enzyme for this conversion. The epimerase was found to be a homodimer of a 28 kDa subunit. From these results, a biosynthesis and metabolism for radicinin was deduced as shown in Fig. 1. First, deoxyradicinin is converted to radicinin by deoxyradicinin monooxygenase, and then, radicin

in epimerase catalyzes epimerizati on of radicinin at C-3 to 3-*epi*-ra dicinin. Finally, 3-*epi*-radicinin is probably converted to 3-*epi*-radici nol by stereospecific reduction at C-4, followed by epoxidation of the side chain in 3-*epi*-radicinol.

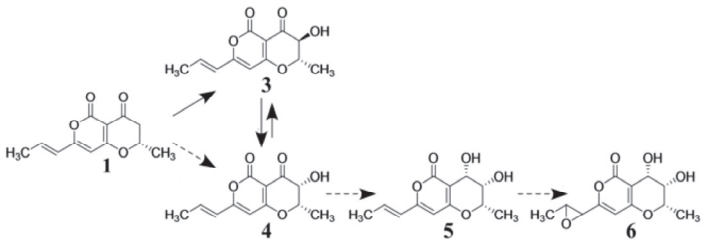
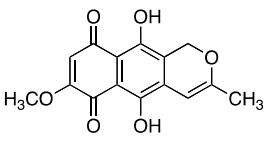
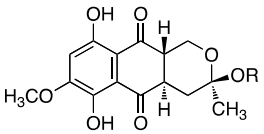


Fig. 1

The fungus *Fusarium* is a well-known soil-borne saprophytic and parasitic fungus that produces diverse bioactive secondary metabolites. *Fusarium* sp. Mj-2, isolated from a soil sample collected in Tottori Prefecture was cultured on a malt extract medium, and the metabolites in the culture f iltrate were extracted with EtOAc. The extract was purified by chromatographic separations to give compounds **11**–**16**. The spectroscopic data of compound **11** agreed well with the reported data fo r anhydrofusarubin. The structures of compounds **11**-**16** were elucidated by spectroscopic analyses t o be 3-*O*-butyl (**12**), 3-*O*-3'-methylbutyl (**13**), 3-*O*-2'-methylbutyl (**14**) and 3-*O*-2'-phenylethyl-4a,10 a-dihydrofusarubin (**15**), and an isomer of the 3-*O*-2'-phenylethyl-4a,10a-dihydrofusarubin (**16**) (Fig. 2). Based on ^{1}H-^{1}H coupling constants and NOESY correlations, the relative stereochemistry of c ompounds **12**-**15** was determined to be 3*R**, 4a*R**, l0a*S**, and that of 3-*O*-methyl-4a,10a-methyldih ydrofusarubin A (**17**), prepared from 3-*O*-butyl-4a,10a-dihydrofusarubin A (**12**), which was reported previously as 3*S**, 4a*R**, 10a*S** should be revised to be 3*R**, 4a*R**, l0a*S**. Their antifungal and a ntibacterial activities were evaluated together with 3-*O*-methyl derivative (**17**) using the fungi, *Mag naporthe grisea*, *Aspergillus oryzae* and *Penicillium citrinum*, and the Gram-positive bacteria *Bacill us subtilis* and *Staphylococcus aureus*, and Gram-negative bacteria *Escherichia coli* and *Pseudomon as aeruginosa*,. Among the compounds, compound **11** exhibited the most potent, but moderate to weak activity against the fungi and the bacteria. Compounds **17** and **12** also exhibited moderate to weak antifungal activities and weak antibacterial activity, but compounds **13**–**16** did not show any antifungal activity against three fungi tested and any antibacterial activity, except for weak antiba cterial activity only against *S. a ureus* of both compounds **13** an d **14**. These results indicated th at the size of the *O*-substituent at C-3 in the 4a,10a-dihydrofusa rubins negatively affects the me tabolites' antimicrobial activity.



11



12 R=$CH_2CH_2CH_2CH_3$ **13** R=$CH_2CH_2CH(CH_3)_2$
14 R=$CH_2CH(CH_3)CH_2CH_3$ **15** R=$CH_2CH_2C_6H_5$
17 R=CH_3

Fig. 2

In conclusion, biosynthesis and metabolism of phytotoxin radicinin in phytopathogen *Bipolaris c oicis* H13-3 was studies and the deoxyradicinin monooxygenase that catalyzed conversion of deoxy radicinin to radicinin and the radicinin epimerase that catalyzed the conversion of radicinin to 3-*ep i*-radicinin were characterized. The five new 3-*O*-alkyl-4a,10a-dihydrofusarubins were isolated from the soil-born phytopathogen *Fusarium* sp. Mj-2 and their antimicrobial activities were evaluated. T he present study provided insight into the function and role of the secondary metabolites produced by the phytopathogenic fungi in the pathogenesis.