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Short communication

Ultraviolet-induced amides and casbene diterpenoids from rice leaves

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ABSTRACT

To discover new phytoalexins, an 80% MeOH extract of UV-irradiated rice leaves was analyzed using LC–MS, resulting in the detection of three unidentified compounds. We isolated the compounds from the UV-irradiated rice leaves using chromatographic methods and identified the compounds as *N*-benzoyltyramine (**1**), and two casbene-type diterpenes, 5-dihydro-*ent*-10-oxodepresssin (**2**) and 5-deoxo-*ent*-10-oxodepressin (**3**), using spectroscopic methods. Additionally, we compared the accumulation levels of major UV-inducible compounds in response to *Magnaporthe oryzae* inoculation and the antifungal activities of the compounds against *M. oryzae* colony growth. Although **1–3** showed negligible antifungal activity against *M. oryzae*, the compounds significantly accumulated in *M. oryzae*-inoculated rice leaves. Furthermore, we confirmed that *N*-benzoyltryptamine and *N*-cinnamoyltryptamine also accumulated after *M. oryzae* inoculation and have relatively high antifungal activity against *M. oryzae* to the same extent as phytocassanes. These results strongly support the hypothesis that the two amides are rice phytoalexins.

1. Introduction

Phytoalexins are low-molecular-weight organic compounds that exhibit antimicrobial activity against pathogens and are biosynthesized de novo after abiotic or biotic stresses such as microbial infection (Ahuja et al., 2012). To date, 16 diterpenoids and one flavonoid, sakuranetin (**4**), have been identified as phytoalexins from rice leaves (Yamane, 2013; Inoue et al., 2013; Horie et al., 2015). The diterpene phytoalexins have been further classified into five types according to their structures: pimaradiene-type, momilactones A (**5**) and B (**6**); stemarene-type, oryzalexin S (**7**); *ent*-cassadiene-type, phytocassanes A–F (**8**–**13**); *ent*-sandaracopimaradiene-type, oryzalexins A–F (numbered as follows: A, **14**; C, **15**; E, **16**; F, **17**); casbene-type, *ent*-10-oxodepressin (**18**, Fig. 1).

The biosynthesis of labdane-related diterpene phytoalexins has been extensively investigated (Yamane et al., 2013). Their hydrocarbon intermediates are biosynthesized from geranylgeranyl diphosphate, by dual cyclizations via *syn*- or *ent*-copalyl diphosphate. These aliphatic and inactive intermediates have been considered to be oxidized by cytochrome P450 or dehydrogenase to produce the bioactive phytoalexins. However, the biosynthesis of the casbene-type diterpene phytoalexin, **18**, is still unclear.

UV irradiation has been used to isolate and identify rice phytoalexins because of its ease and convenience. We confirmed that all rice phytoalexins accumulate in response to fungal infection and UV irradiation (Kodama et al., 1988; Horie et al., 2015). Recently, Park et al. (2013, 2014) reported several amide compounds, *N*-benzoyltryptamine (**19**), *N*-cinnamoyltryptamine (**20**) and *N*-cinnamoyltyramine (**21**), from UV-irradiated rice leaves. They showed that these compounds have antimicrobial activity against *Bipolaris oryzae* and *Xanthomonas oryzae* pv. *oryzae*. However, the induction of their biosynthesis in response to infection has still not been confirmed.

In this study, we comprehensively analyzed unidentified UV-inducible compounds from rice leaves using an LC–MS technique followed by complementary spectroscopic techniques and chemical synthesis. Furthermore, we investigated the accumulation of these compounds in response to *M. oryzae* inoculation and

Abbreviations: EMS, enhanced mass spectrometry; EPI, enhanced product ion; SRM, selective reaction monitoring; PDA, potato dextrose agar; CUR, curtain gas; TEM, temperature; GS1, spray gas; GS2, dry gas; IS, ion voltage; DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potensial; CE, collision energy; CAD, collision gas.

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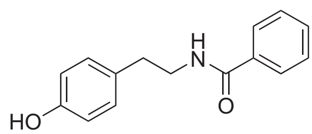

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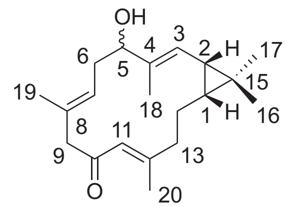

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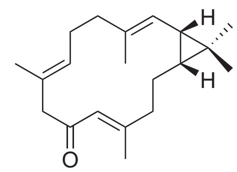

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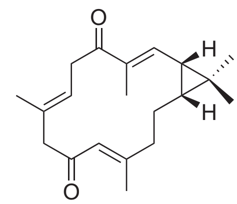

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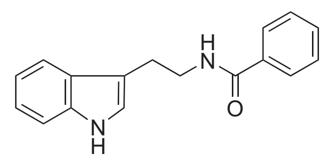

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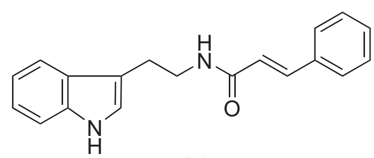

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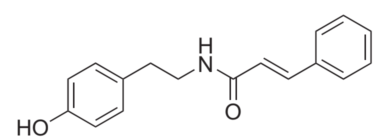

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Fig. 1. Compound structures. **1**,*N*-benzoyltyramine; **2**, 5-dihydro-*ent*-10-oxodepressin; **3**, 5-deoxo-*ent*-10-oxodepressin; **18**, *ent*-10-oxodepressin; **19**, *N*-benzoyltryptamine; **20**, *N*-cinnamoyltryptamine; **21**, *N*-cinnamoyltyramine.

compared the antifungal activity of rice phytoalexins against *M. oryzae*.

2. Results and discussion

We investigated whether major and unidentified UV-inducible compounds could be detected in UV-irradiated rice leaves. Previously, we discovered phytocassane F in UV-irradiated rice leaves using an LC–MS method (Horie et al., 2015). In this study, we added an EtOAC–water partitioning procedure to the sample preparation, and LC separation was improved by using MeCN instead of MeOH as an eluent. The extracts from control and UV-irradiated rice leaves were subjected to LC–MS analysis in enhanced MS (EMS) scan mode. The t_R and mass chromatograms of UV-inducible compounds were compared with those of phytoalexin standards to assign peaks to known compounds

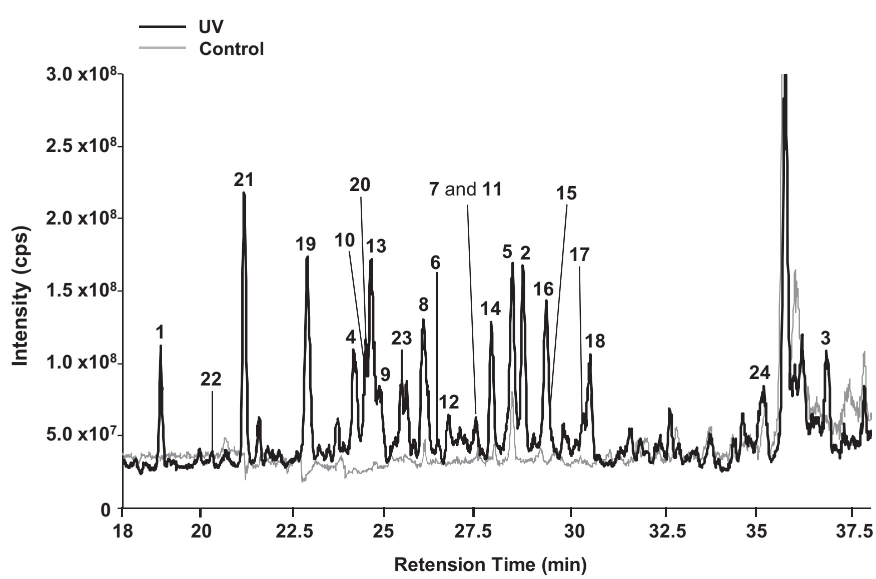

Fig. 2. Total ion current chromatograms obtained from UV-irradiated and control rice leaves using LC–MS. **4**, sakuranetin; **5**, momilactone A; **6**, momilactone B; **7**, oryzalexin S; **8**, phytocassane A; **9**, phytocassane B; **10**, phytocassane C; **11**, phytocassane D; **12**, phytocassane E; **13**, phytocassane F; **14**, oryzalexin A; **15**, oryzalexin C; **16**, oryzalexin E; **17**, oryzalexin F; **18**, *ent*-10-oxodepressin; **19**, *N*-benzoyltryptamine; **20**, *N*-cinnamoyltryptamine; **21**, *N*-cinnamoyltyramine; **22**, naringenin; **23**, pimara-7,15-diene-3β,6β,19-triol; **24**, stemar-13-en-2α-ol.