COMMUNICATIONS TO THE EDITOR

Pyridovericin and Pyridomacrolidin: Novel Metabolites from Entomopathogenic Fungi, *Beauveria bassiana*

Sir:

Many entomopathogenic fungi are presumed to cause host death through the inhibition of enzymes and interference with the regulatory system in the host by production of toxic fungal metabolites and pathogenic enzymes[1~3]. The entomopathogenic fungi can be classified into five genera: Oomycota, Chytridiomycota, Zygomycota, Ascomycota, and Deuteromycota, and their hosts are various[4~6]. Among these fungi, Deuteromycota seems to be preferred as a source of screening for new bioactive compounds from fungal metabolites as fungi can be cultured on artificial media. So far, many bioactive compounds with insecticidal, antifungal and immunosuppressive activity have been isolated successfully from entomopathogenic fungal metabolites[7~12]. In the course of our HPLC screening program, we systematically investigated the diversity of metabolites in Deuteromycota, *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces fumosoroseus* and *Verticillium lecanii*, which have been isolated from the corpses of insects. Comparative study of the HPLC elution pattern among fungal metabolites found that a strain, *Beauveria bassiana* EPF-5, produced two novel metabolites, designated pyridovericin (**1**) and pyridomacrolidin (**2**). In this communication, we preliminarily report the production, isolation, structure and biological activities of **1** and **2**.

The detection of fungal metabolites by HPLC analysis was performed under the following conditions: column: ODS column (Senshu Pak PEGASIL); solvent: a linear gradient 50% MeOH to 70% MeOH for 50 minutes; UV detection at 220 nm, 254 nm and 310 nm; flow rate of 3.0 ml/minute. *Beauveria bassiana* EPF-5, a strain isolated from an adult Mulberry small weevil (*Baris deplanata* Roelofs) was cultured at 27°C for 5 days in a 500-ml Erlenmeyer flasks containing 100 ml of the CzS-8 medium [glucose 1.5%, saccharose 1.5%, soybean powder 1.0% (Honen Co.), KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05%, $FeSO_4 \cdot 7H_2O$ 0.001%, $CaCO_3$ 0.5%, pH 6.0]. The fermentation broth (18 liters) was centrifuged and the resulting mycelial cake was extracted with acetone. After removal of the acetone, the aqueous solution was extracted twice with ethyl acetate. The extracts was concentrated *in vacuo* to dryness to give a brownish oil (15.2 g). The oily substance was applied to a silica gel column and eluted with a solvent system of chloroform-methanol to give a crude powder (268 mg). The powder was further purified by preparative HPLC (Senshu Pak ODS H-5251) with 73% methanol and repeat HPLC (Senshu Pak PEGASIL ODS) developing with 70% methanol to give **1** (41 mg) and **2** (34 mg), as a pale yellowish powder.

The molecular formulas, $C_{21}H_{23}NO_5$ for **1** and $C_{31}H_{35}NO_{10}$ for **2**, were established by high-resolution EI-MS and high-resolution FAB-MS measurements [Found *m/z* 369.1580 (M^+), Calcd for $C_{21}H_{23}NO_5$ 369.1576 for **1**; Found *m/z* 582.2329 $(M+H)^+$, Calcd for $C_{31}H_{36}NO_{10}$ 582.2339 for **2**], which were supported by ^1H and ^{13}C NMR spectral data. ^1H NMR spectra (DMSO-d_6) of **1** and **2** are shown in Fig. 1. The physico-chemical properties of **1** and **2** were as follows, **1**: MP 203~206°C (dec.); $[\alpha]_D^{15}$ −20.3° (*c* 0.1, MeOH); UV λ_{max} in MeOH (ε) 248 nm (16,400) and 336 nm (15,900); **2**: MP 192~194°C (dec.); $[\alpha]_D^{15}$ +19.4° (*c* 0.1, MeOH); UV λ_{max} in MeOH (ε) 238 nm (14,500) and 342 nm (14,600). Both compounds are soluble in methanol, ethyl acetate, acetone and dimethyl sulfoxide, but insoluble in *n*-hexane and water. The ^{13}C NMR and DEPT spectra of both compounds revealed the presence of $CH_3 \times 2$, $CH_2 \times 1$, $OCH_2 \times 1$, $CH \times 1$, $=CH \times 8$, $=C \times 6$, $C=O \times 2$ for **1** and $CH_3 \times 3$, $CH_2 \times 4$, $OCH_2 \times 1$, $CH \times 2$, $OCH \times 3$, $=CH \times 7$, $=C \times 7$, $C=O \times 4$ for **2**. The structures of compounds **1** and **2** were deduced from 2D NMR experiments including COSY, ROESY and HMBC, as shown in Fig. 2. Compounds, **1** and **2** have a 4-hydroxy-5-(*p*-hydroxyphenyl)pyridone ring with a dienone unit consisting of $C_{10}H_{15}O_2$ as a common chromophore. From comparison of the NMR spectral data of **1** and **2**, it was found that compound **2** possesses a novel 10-membered ring macrocyclic structure with $C_{10}H_{14}O_5$ connected to a pyridone ring of compound **1**. The ^1H and ^{13}C chemical shift assignments for **1** and **2** are indicated below, **1**; C-2 (δ_C 161.7), C-3 (δ_C 105.9), C-4 (δ_C 176.9), C-4-OH (δ_H 17.54), C-5 (δ_C 112.7), C-6 (δ_C 140.5/δ_H 7.54), C-7 (δ_C 193.7), C-8 (δ_C 123.1/δ_H 8.00), C-9 (δ_C 149.3/δ_H 7.51), C-10 (δ_C 134.5), C-11 (δ_C 147.4/δ_H 5.95), C-12 (δ_C 43.5/δ_H 2.53), C-13 (δ_C 24.0/δ_H 1.22, 1.59),

Fig. 1. ^{1}H-NMR spectra of pyridovericin (**1**) and pyridomacrolidin (**2**) in DMSO-d_6 (400 MHz).

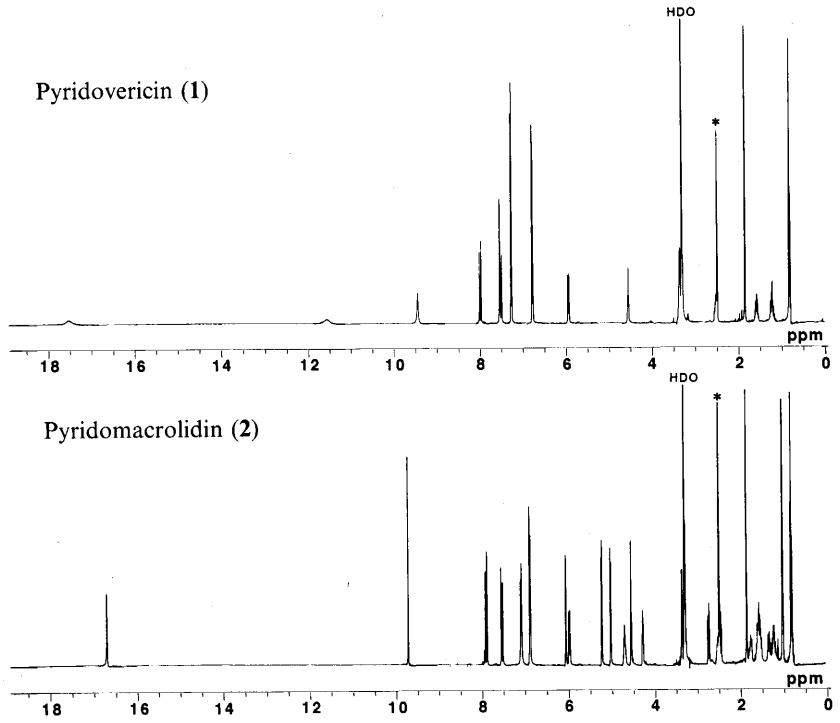
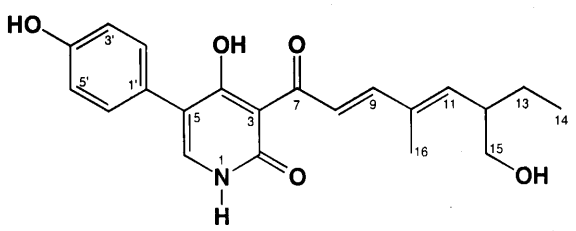
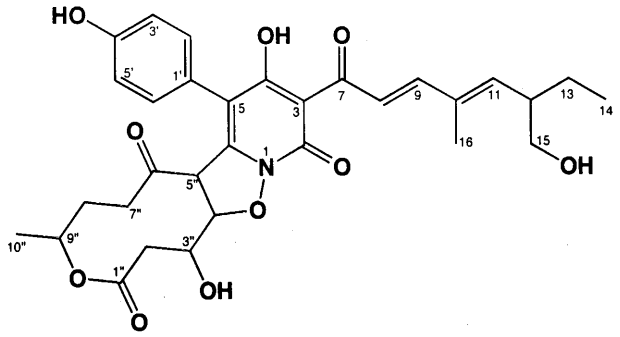


Fig. 2. The structures of pyridovericin (**1**) and pyridomacrolidin (**2**).



Pyridovericin (**1**)



Pyridomacrolidin (**2**)

C-14 (δ_C 11.6/δ_H 0.82), C-15 (δ_C 64.0/δ_H 3.37), C-15-OH (δ_H 4.56), C-16 (δ_C 12.7/δ_H 1.85), C-1′ (δ_C 123.4), C-2′ and 6′ (δ_C 130.0/δ_H 7.27), C-3′ and 5′ (δ_C 114.9/δ_H 6.78), C-4′ (δ_C 156.7), C-4′-OH (δ_H 9.45), NH-1 (δ_H 11.58), **2**; C-2 (δ_C 153.1), C-3 (δ_C 105.6), C-4 (δ_C 173.7), C-4-OH (δ_H 16.71), C-5 (δ_C 106.5), C-6 (δ_C 145.0), C-7 (δ_C 193.1), C-8 (δ_C 122.6/δ_H 7.90), C-9 (δ_C 149.9/δ_H 7.53), C-10 (δ_C 134.4), C-11 (δ_C 148.1/δ_H 5.98), C-12 (δ_C 43.6/δ_H 2.54), C-13 (δ_C 23.9/δ_H 1.24, 1.58), C-14 (δ_C 11.6/δ_H 0.83), C-15 (δ_C 64.0/δ_H 3.37), C-15-OH (δ_H 4.56), C-16 (δ_C 12.7/δ_H 1.86), C-1′ (δ_C 121.5), C-2′ and 6′ (δ_C 131.6/δ_H 7.09), C-3′ and 5′ (δ_C 115.7/δ_H 6.88), C-4′ (δ_C 157.7), C-4′-OH (δ_H 9.72), C-1″ (δ_C 168.4), C-2″ (δ_C 40.7/δ_H 2.47, 2.75), C-3″ (δ_C 67.5/δ_H 4.29), C-3″-OH (δ_H 6.06), C-4″ (δ_C 87.8/δ_H 5.03), C-5″ (δ_C 54.7/δ_H 5.23), C-6″ (δ_C 207.4), C-7″ (δ_C 39.8/δ_H 1.36, 1.58), C-8″ (δ_C 32.8/δ_H 1.58, 1.77), C-9″ (δ_C 71.4/δ_H 4.71), C-10″ (δ_C 18.5/δ_H 1.03). Details of the structure elucidation of **1** and **2** will be reported later.

Tenellin, bassianin[13,14] and ilicicolin H[15], which are related compounds with a 4-hydroxy-5-(*p*-hydroxyphenyl)pyridone skeleton have been isolated from *Beauveria tenella*, *Beauveria bassiana* and *Cylindrocladium ilicicola*, respectively. Although ilicicolin H is known to possess antifungal activity, there has been no report concerning the biological activities of tenellin and bassianin. Compounds **1** and **2** did not exhibit antimicrobial activity against typical Gram-positive (*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis* and *Mycobacterium smegmatis*) and Gram-negative bacteria (*Escherichia coli*

and *Pseudomonas aeruginosa*, fungi (*Aspergillus niger*, *Pyricularia oryzae*, *Mucor racemosus* and *Candida albicans*) or *Saccharomyces sake* at the concentration of 100 μg/ml by the paper disc method. Both compounds weakly inhibited protein tyrosine kinase at the concentration of 100 μg/ml in an assay system containing multiple protein kinase activities in a crude cell homogenate prepared from NIH3T3/v-src[16].

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