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ISOFUNICONE, A POLLEN GROWTH INHIBITOR PRODUCED BY THE FUNGUS, *PENICILLIUM* SP.

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Key Word Index—*Penicillium* sp.; fungus; isofunicone; tea pollen; growth inhibitor.

Abstract—Isofunicone, a new metabolite from an unidentified *Penicillium* sp.; was shown to be (*E*)-3-methoxy-2-propenyl-5-(2′-carbomethyoxy-4′-hydroxy-6′-methoxybenzoyl)-4-pyrone. It inhibited tea pollen (*Camellia sinensis* O. Kuntze) growth by 84% at a concentration of 3 mg l^{-1}, and completely at a concentration of 10 mg l^{-1}.

INTRODUCTION

We have investigated fungal metabolites as pollen growth inhibitors by means of the bioassay method using tea pollen grains of *Camellia sinensis* O. Kuntze [1], because such inhibitors may be useful for developing new herbicides and as tools to analyse the reproductive functions in higher plants [2–4]. So far, we have isolated and characterized naphthoquinones [5], vulculic acid [6], hericerine [7] and emeniveol [8] as pollen growth and germination inhibitors.

In this paper, we describe the isolation and structural determination of isofunicone (**1**) a growth inhibitor of tea pollen, from the culture filtrate of a *Penicillium* sp. Isofunicone is an analogue of funicone produced by the fungus, *Penicillium funiculosum* Thom [9].

RESULTS AND DISCUSSION

Isofunicone (**1**) was isolated from the culture filtrate as 21-day-old stationary cultures of a *Penicillium* sp. Its molecular formula as determined by EI mass spectroscopy ($[M]^+$ *m/z* 374) and confirmed by elementary analysis (Found: C, 66.99; H, 4.75. Calcd: C, 60.96; H, 4.85%) was shown to be $C_{19}H_{18}O_8$. This formula indicated 11 double bond equivalents. The presence of an aromatic ring in the molecule was suggested by an IR band at 1611 cm^{-1}. A band at 3228 cm^{-1} indicated the presence of a phenolic hydroxyl group, which was positive to alcoholic ferric chloride, while three bands at 1715, 1681 and 1653 cm^{-1} provided evidence for carbonyl groups.

The IR, ^1H NMR and ^{13}C NMR spectra of **1** showed several partial structures: the signals at δ_H3.64 (3H, *s*), 3.66 (3H, *s*) and 3.71 (3H, *s*) were assigned to the methoxyl groups, respectively. The two ethylenic protons (δ6.54 and 6.62) were is the *E* configuration as indicated by their mutual coupling constants (J = 15.0 Hz). Since the ethylenic proton at δ6.62 was linked to the methyl group (δ 1.94), as indicated by their mutual coupling constants (J = 5.0 Hz), a propenyl group was indicated. The two aromatic protons (δ6.64 and 6.81) were in the *meta* relationship as indicated by their mutual coupling constants (J = 2.0 Hz). The signal at δ10.21 was assigned to the phenolic hydroxyl group mentioned above. The presence of a γ-pyrone ring in the molecule was suggested by an IR band at 1653 cm^{-1} and a single at δ_C171.1. Since the signal at δ85.1 assignable to a methine proton appeared at δ159.0 in the ^{13}C NMR spectrum, this was assigned to the methine proton in the α-position in the γ-pyrone ring.

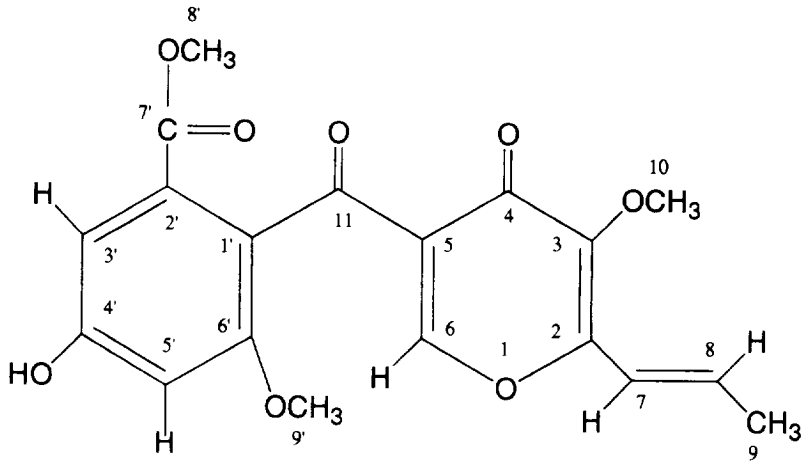
From the results of the measurement of the ^1H–^{13}C long range couplings (Fig. 1), the propenyl group had to placed at C-2 (δ153.7), and the methoxyl group (δ_H3.64) was linked to C-3 (δ157.9). On the other hand, the methoxyl group (δ_H3.66) was linked to a carbonyl group (δ166.3) adjacent to C-2′ (δ130.6). Furthermore, the hydroxyl group (δ_C10.21) was linked to C-4′ (δ159.3) adjacent to C-3′ and C-5′ (δ107.7 and 102.9), and the methoxyl group (δ_H3.71) was linked to the C-6′ carbon (δ143.2). The remaining carbonyl carbon (δ189.7 and 1681 cm^{-1}) was bound to the two quaternary carbons (C-5 and C-1′). Thus, the structure of isofunicone was established to be (*E*)-3-methoxy-2-propenyl-5-(2′-carbomethoxy-4′-hydroxy-6′-methoxybenzoyl)-4-pyrone (**1**).

As shown in Fig. 2, **1** inhibited tea pollen growth by 84% at a concentration of 3 mg l^{-1}, and completely at 10 mg l^{-1}.

EXPERIMENTAL

General. IR: KBr pellet; NMR; 270.05 MHz (^1H) and 67.80 MHz (^{13}C) in DMSO with TMS as int. standard; EIMS: 70 eV; CC: silica gel (Grade C-200) Wako.

Bioassay for pollen growth. Pollen grains of *C. sinensis* O. Kuntze were collected from an open flower, dried in a desiccator over silica gel and stored in a refrigerator. The grains were sown in a straight line (by means of



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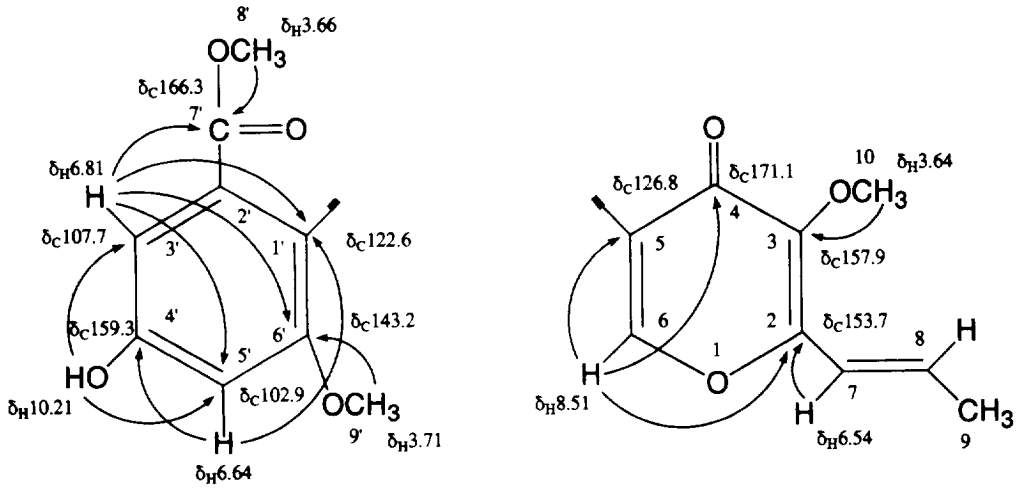

Fig. 1. $^{1}H-^{13}C$ long range couplings of isofunicone.

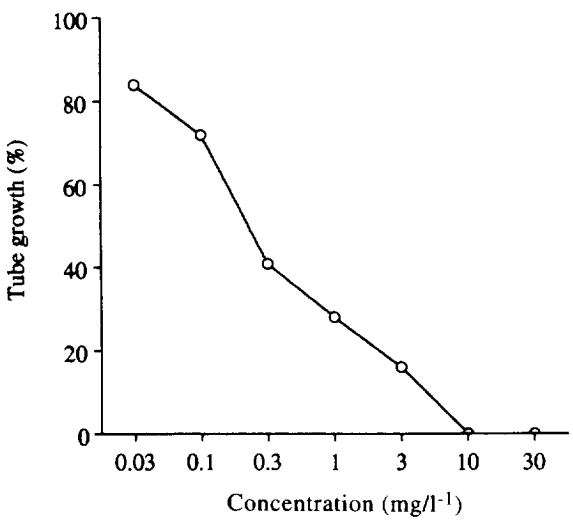

Fig. 2. Effect of isofunicone on tea pollen growth.

a cover glass) on a 1.5% agar medium containing 10% sucrose, 0.002% B as H_3BO_3 and the compound to be tested at various concns on a micorscopic slide, and then incubated in a moist chamber at 24°C in the dark. After cultivating for 12 hr, the length of tea pollen tubes was measured and compared with that of an untreated control.

Screening of fungal metabolites. The fungus was cultured without shaking in a malt extract medium containing glucose (50 g l^{-1}) and peptone (3 g l^{-1}) at 24° for 21 days. The culture broth was then filtered, the filtrate was adjusted to pH 2.0 with dil. HCl soln and extracted with EtOAc. The mycelial mats were extracted with Me_2CO. After evaporating the solvents, both extracts were assayed at a concn of 300 mg/.

Extraction and isolation of **1**. One hundred and sixty, 500 ml Erlenmeyer flasks, each containing 250 ml malt extract medium made from malt extract (20 g l^{-1}), glucose (50 g l^{-1}) and peptone (3 g l^{-1}), were inoculated with

spores of *Penicillium* sp. previously grown on solid potato dextrose agar. The culture broth (40 l) was grown at 24° without shaking for 21 days and then filtered to separate the mycelium from the broth. The filtrate was adjusted to pH 2.0 with dil. HCl soln, and successively extracted with EtOAc. After evaporating the solvent, the residue (19.5 g) was fractionated by silica gel CC with hexane–EtOAc mixts. Frs containing 50% EtOAc (12–15:2482.2 mg) were bulked and further purified by silica gel CC with C_6H_6–EtOAc mixts. The active fr. (381.1 mg) was evapd to dryness and recrystallized from MeOH, to give plates of **1** (20 mg).

Isofunicone (**1**). Plates, mp 215–218°. $C_{19}H_{18}O_8$ (M_r 374). UV λ_{max}^{EtOH} nm: 293, 285, 249; IR ν_{max}^{KBr} cm^{-1}: 3228, 2950, 2852, 1715, 1681, 1653, 1611, 1443; ^{1}H NMR (270.05 MHz, DMSO): δ 1.94 (3H, *s*, H-9), 3.64 (3H, *s*, H-10), 3.66 (3H, *s*, H-8′), 3.71 (3H, *s*, H-9′), 6.54 (1H, *br. d*, J = 15 Hz, H-7), 6.62 (1H, *m*, H-8), 6.64 (1 H, *d*, J = 2 Hz, H-5′), 6.81 (1H, *d*, J = 2 Hz, H-3′), 8.51 (1H, *s*, H-6), 10.21 (1H, *br. s*, H-4′); ^{13}C NMR (67.80 MHz, DMSO): δ 18.4 (*q*, C-9), 52.0 (*q*, C-8′), 55.9 (*q*, C-10), 60.0 (*q*, C-9′), 102.9 (*d*, C-5′), 107.7 (*d*, C-3′), 118.1 (*d*, C-8), 122.6 (*s*, C-1′), 126.8 (*s*, C-5), 130.6 (*s*, C-2′) 135.1 (*d*, C-7), 143.2 (*s*, C-6′), 153.7 (*s*, C-2), 157.9 (*s*, C-3), 159.0 (*d*, C-6), 159.3 (*s*, C-4′), 166.3 (*s*, C-7′), 171.1 (*s*, C-4), 189.7 (*s*, C-11); EIMS (probe) 70 eV, *m/z* (rel. int.): 374 $[M]^+$ (82), 343 $[M - OMe]^+$ (39), 315 $[M - COOMe]^+$ (42), 209 $[M - 165]^+$ (40), 192 $[M - 182]^+$ (100). Found: C, 60.99; H, 4.75. $C_{19}H_{18}O_8$ requires: C, 60.96; H, 4.85%.

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