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PENIDIENONE, A PLANT GROWTH REGULATOR PRODUCED BY THE FUNGUS, *PENICILLIUM* SP. NO. 13

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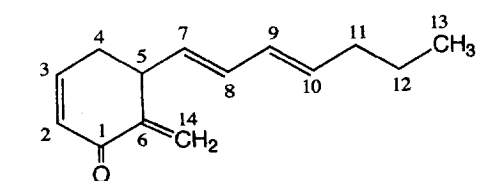
Key Word Index—*Penicillium* sp.; fungus; penidienone; plant growth inhibitor.

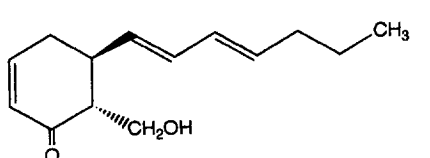
Abstract—Penidienone was isolated from the fungus, *Penicillium* sp. No. 13, and its structure was established by NMR studies. The compound inhibited the growth of lettuce and rice seedlings, and germination of pine pollen.

INTRODUCTION

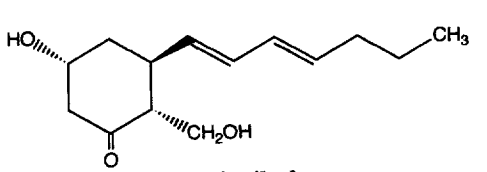
We have elucidated the structures of botryslactone [1] and cyclo-(L-tryptophyl-L-phenylalanyl) [2] which regulate the growth of lettuce seedlings. In addition, we found penienone and penihydrone [3] from *Penicillium* sp. No. 13 to be growth regulators of lettuce and rice seedlings (Fig. 1). In our continuing search for compounds from the metabolites of *Penicillium* sp. No. 13 which regulate plant growth, we identified



1 : Penidienone



Penienone



Penihydrone

Structure 1.

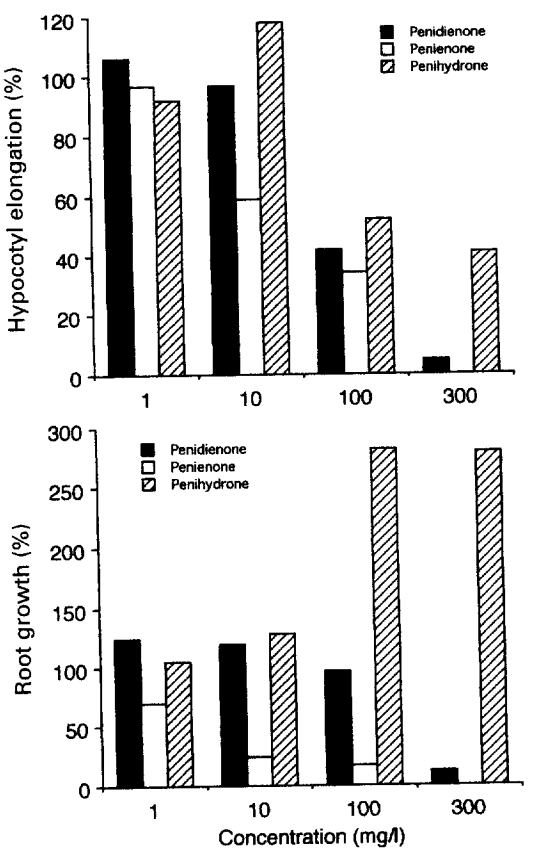


Fig 1. Effect of **1**, penienone and penihydrone on the growth of lettuce seedlings.

penidienone (**1**). Here we describe the structural elucidation and the biological activities of **1** together with those of penienone and penihydrone.

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RESULTS AND DISCUSSION

Penidienone (**1**) was isolated from the culture filtrate of a 21-day-old stationary culture of *Penicillium* sp. No. 13 in a potato extract medium (see Experimental for details). The molecular formula of **1** was established as $C_{14}H_{18}O$ by HREI-mass spectrometry ($[M]^+$ observed, m/z 202.1356, calculated, 202.1356). The molecular formula indicated six degrees of unsaturation. In the ^1H NMR data for **1**, one methyl signal at δ_H 0.94 (H-13), three methylene signals at δ_H 1.45 (H-12), 2.10 (H-11) and 2.40, 2.62 (H-4), one methine signal at δ_H 3.50 (H-5) and eight olefinic proton signals at δ_H 5.34 (H-14), 5.63 (H-7), 5.72 (H-10), 6.08 (H-14), 6.11 (H-8,9), 6.20 (H-2) and 7.01 (H-3) were observed. The IR absorption band at 1673 cm^{-1} and one signal at δ_C 188.7 (*s*) indicated the presence of an α,β-unsaturated carbonyl carbon. The conjugated diene at δ_H 5.63 (H-7) and 6.11 (H-8), and at δ_H 5.72 (H-10) and 6.11 (H-9) indicated the *E*-geometry by their coupling constant (J = 14.2 Hz). On the other hand, the coupling constants (J = 10.3 Hz) of two olefinic protons at δ_H 6.20 (H-2) and 7.01 (H-3) showed the *Z*-geometry. From these results, together with those of Homospin-decoupling experiments, the presence of the partial structure **A** was derived. The presence of one exomethylene groups of **B** was indicated by an IR absorption band at 1735 cm^{-1} together with signals at δ_H 5.34 (H-14) and 6.08 (H-14) in the ^1H NMR data and signals at δ_C 120.9 (*t*) and 145.7 (*s*) in the ^{13}C NMR data. Since the two partial structures accounted for all the atoms of **1**, the planar structure of **1** was established to be 5-[(1*E*, 3*E*)-1,3-heptadienyl-6-methylene-2-cyclohexenone and the compound was named penidienone.

Compound **1**, penienone and penihydrone, which had been isolated from the same fungus, were tested using the same plants in order to compare the respective structure–activity relationship. Compound **1** inhibited hypocotyl elongation and root growth of lettuce seedlings [4] by 95% and 88% at a concentration of 300 mg l^{-1}, respectively (Fig. 1). On the other hand, penienone completely inhibited hypocotyl elongation and root growth at the same concentration. Penihydrone inhibited the hypocotyl elongation by 59%, but accelerated root growth by 280% at the same concentration. Compound **1** did not show any remarkable inhibitory activity against growth of the total and the second leaf sheath length of rice seedlings [5], but inhibited growth of the primary root by 84% at a concentration of 300 mg l^{-1} (Fig. 2). Penienone completely inhibited growth of the total length, the second leaf sheath and the primary root at the same concentration. Penihydrone did not show inhibitory activities against growth of the total length and the second leaf sheath at the same concentration, but inhibited growth of the primary root by 95% at the same concentration. Compound **1** and penienone inhibited pine pollen germination [6] by 91 and 95% at a concentration of 100 mg l^{-1}, respectively (Fig. 3). On the other hand, penihydrone only weakly inhibited pine pollen germination at the same concentration, but showed almost complete inhibition at a concentration of 300 mg l^{-1}. From these results, a conjugated double bond in a cyclohexenone ring may play an important role in promoting inhibitory activity.

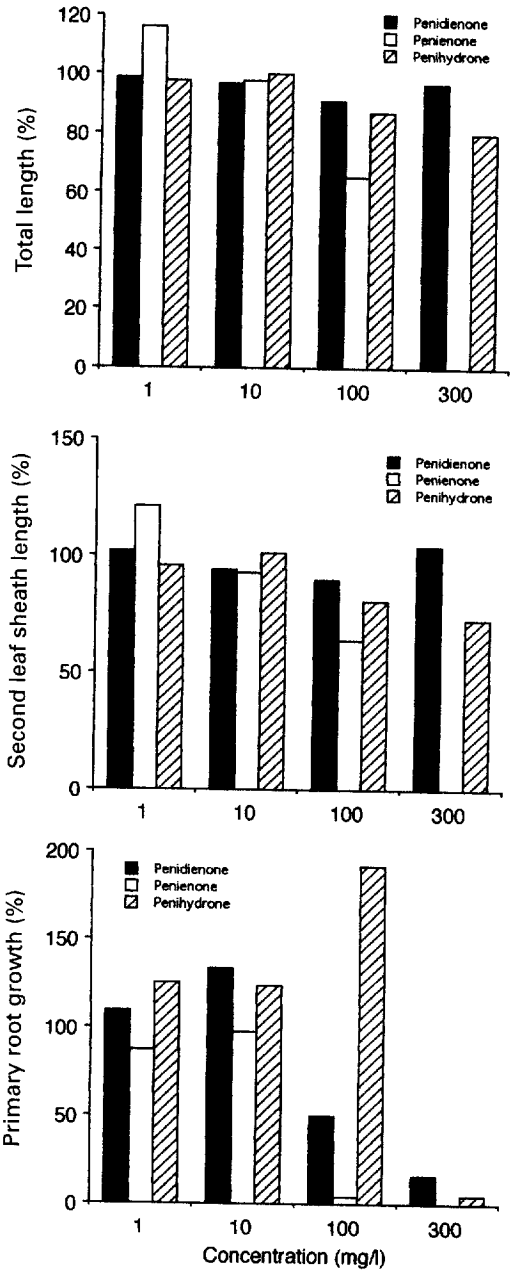
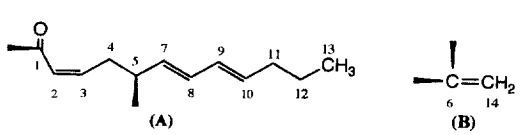


Fig 2. Effect of **1**, penienone and penihydrone on the growth of rice seedlings.



Structure 2.

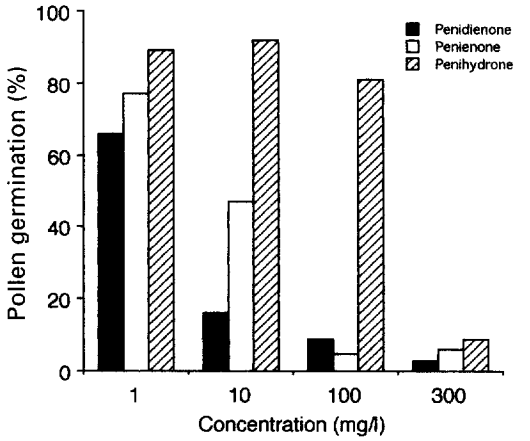


Fig 3. Effect of **1**, penienone and penihydrone on pine pollen germination.

EXPERIMENTAL

General. Optical rotation was measured with a YANACO OR-50 digital polarimeter. UV spectra were recorded with a Hitachi 100-50 spectrometer. IR spectra were recorded with a Jasco FT/IR-7000 spectrometer. The M_r was determined by HREIMS spectroscopy using a Hitachi M-80. ^{1}H (270.05 MHz) and ^{13}C NMR (67.80 MHz) spectra were recorded with a JEOL JNM-GX270 spectrometer. CC was performed with silica gel (Wakogel C-200) using the indicated solvent system.

Isolation and purification of penidienone. Penicillium sp. No. 13 was cultured by stationary culture in a potato medium containing sucrose (30 g l^{-1}) at 24° for 21 days. The culture broth (10 l.) was filtered and the filtrate was adjusted to pH 2.0 with dilute HCl soln. Subsequently, the filtrate was successively extracted with EtOAc. After evaporating the solvent, the residue (2.2 g) was purified by CC (*n*-hexane–EtOAc) on silica gel. The fr. eluted with *n*-hexane–EtOAc (49:1) was purified by prep. TLC on Kieselgel 60 GF_{254} eluting with C_6H_6 and evapd to afford **1** (29 mg).

Penidienone. Oil. $[\alpha]_D^{20}$ −98.5° (MeOH: *c* 0.65). UV λ_{max}^{EtOH} nm (log ε): 231 (3.87). IR ν_{max}^{KBr} cm^{-1}: 2928, 1735, 1673, 1461, 1393, 1270, 1158, 991, 944. HREIMS *m/z* 202.1356 $[M]^+$, (*m/z* 202.1356 calcd for $C_{14}H_{18}O$). ^{1}H NMR (270.05 MHz, $CDCl_3$): δ 0.94 (3H, *t*, *J* = 7.3 Hz, H-13), 1.45 (2H, *tq*, *J* = 7.3, 7.3 Hz, H-12), 2.10 (2H, *dt*, *J* = 7.3, 6.8 Hz, H-11), 2.40 (1H, *ddd*, *J* = 18.1, 7.8, 3.6 Hz, H-4), 2.62 (1H, *ddd*, *J* = 18.1, 5.7, 4.5 Hz, H-4), 3.50 (1H, *m*, H-5), 5.34 (1H, *s*, H-14), 5.63 (1H, *dd*, *J* = 14.2, 6.8 Hz, H-7), 5.72 (1H, *dt*, *J* = 14.2, 6.8 Hz, H-10), 6.08 (1H, *s*, H-14), 6.11 (1H, *m*, H-8), 6.11 (1H, *m*, H-9), 6.20 (1H, *d*, *J* = 10.3 Hz, H-2), 7.01 (1H, *ddd*, *J* = 10.3, 5.7, 3.6 Hz, H-3). ^{13}C NMR (67.80 MHz, $CDCl_3$): δ 13.7 (*q*, C-13), 22.4 (*t*, C-12), 32.6 (*t*, C-4), 34.7 (*t*, C-11), 44.2 (*d*, C-5), 120.9 (*t*, C-14), 130.0 (*d*, C-9), 130.2 (*d*, C-7), 130.5 (*d*, C-2), 132.6 (*d*, C-8), 134.9 (*d*, C-10), 145.7 (*s*, C-6), 148.9 (*d*, C-3), 188.7 (*s*, C-1).

Bioassay for the growth of lettuce and rice seedlings. The growth of lettuce seedlings treated with the compounds was examined by the established method [4]. The lengths of the hypocotyls and roots of the seedlings after treatment were measured and compared with those of an untreated control. The growth of rice seedlings treated with the compounds was measured by the established method [5]. The total length and the lengths of the second leaf sheaths and roots after treatment were measured and compared with those of an untreated control. Each bioassay was performed × 3 and the mean values are shown in Figs 1 and 2.

Bioassay for pollen germination. Pollen grains of *Pinus thunbergii* Parl. were collected from an open flower, dried in a desiccator over silica gel and stored in a refrigerator. The grains were sown with a paintbrush on a 1.5% agar medium containing 10% sucrose and the compound to be tested at various concns on a microscopic slide, and then incubated in a moist chamber at 24° in the dark. After cultivation for 3 days, the number of germinated grains was measured and compared with that of an untreated control [6]. Bioassay was performed × 3 and the mean values are shown in Fig. 3.

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