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Peniamidienone and penidilamine, plant growth regulators produced by the fungus *Penicillium* sp. No. 13

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Abstract

Peniamidienone and penidilamine were isolated from cultures of the fungus *Penicillium* sp. No. 13 as new plant growth regulators and their structures were established by NMR spectroscopic studies. Peniamidienone showed weak inhibition of lettuce seedling growth. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Penicillium sp; Fungus; Peniamidienone; Penidilamine; Growth regulator; Lettuce

1. Introduction

We previously reported that penienone, penihydrone (Kimura, Mizuno & Shimada, 1997) and penidienone (Kimura, Mizuno, Shimada & Kawano, 1998) from *Penicillium* sp. No. 13 can function as growth regulators of lettuce and rice seedlings, there being isolated from the culture filtrate of *Penicillium* sp. No. 13 grown on a potato extract medium. Recently, we changed to Czapek–Dox medium in order to study the production of new plant growth regulators from *Penicillium* sp. No. 13. These studies led to the isolation of peniamidienone and penidilamine. Here we describe the structure elucidation and biological activity of these new metabolites.

2. Results and discussion

Peniamidienone (**1**), penidilamine (**2**) and penienone (**3**) (Kimura et al., 1997) were isolated from the culture filtrate of a 21-day-old stationary culture of *Penicillium* sp. No. 13 in Czapek–Dox medium. The molecular formula of **1** was established as $C_{19}H_{23}NO_3$ by HR-EIMS ($[M]^+$ observed, *m/z* 313.1677, calculated, 313.1677). The molecular formula indicated nine degrees of unsaturation. The IR absorption band at 1662 cm^{-1}, the UV absorption band at 227 nm and one carbon signal (C1) indicated the presence of an α,β-unsaturated carbonyl carbon. The partial structure corresponding to C1–C14 in **1** was identical to subunits found in the structure of penidienone (**4**), based on homospin-decoupling experiments and comparison to data reported for penidienone (**4**) (Kimura et al., 1998). Besides the proton signals of this partial structure, one methyl signal (H19), one methine signal (H18), one olefinic proton signal (H17),

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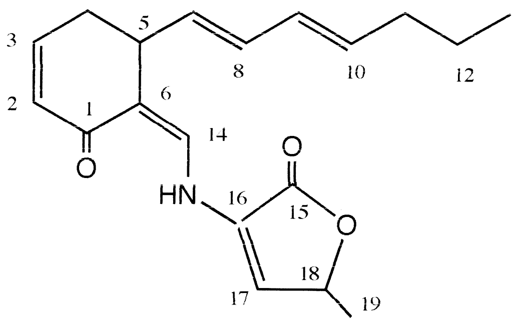
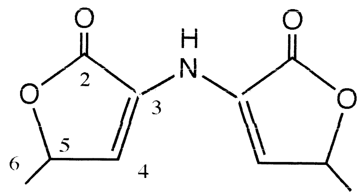
and one D_2O-exchangeable proton signal were observed. The positive reaction to *p*-dimethylaminobenzaldehyde and one D_2O exchangeable proton signal at δ_H 11.0 indicated the presence of a hydrogen-bonded secondary amine. The NH proton was linked to the sp^2 methine (H14) ($J = 12.0$ Hz), indicating the *Z*-geometry resulting from hydrogen bonding to the C1 carbonyl oxygen. The IR absorption band at 1765 cm^{-1}, the UV absorption band at 260 nm and one carbon signal (C15) indicated the presence of an α,β-unsaturated γ-lactone. The methyl group (H19) was linked to the oxygen-bearing methine (H18) ($J = 6.8$ Hz). The olefinic proton (H17) was deduced to be adjacent to the methine group based on their coupling constant ($J = 2.0$ Hz). From these results, the presence of the C15–C19 portion of **1** was derived. Since these partial structures accounted for all the atoms of **1**, the planar structure of **1** was established to be 6-[*N*-(5-methyl-2,5-dihydro-2-oxofuran-3-yl)aminomethylene]-5-[(1*E*, 3*E*)-1,3-heptadienyl]-2-cyclohexenone and the compound was named peniamidienone.

The molecular formula of **2** was established as $C_{10}H_{11}NO_4$ by HR-EIMS ($[M]^+$ observed, m/z 209.0688, calculated, 209.0687). The molecular formula indicated six degrees of unsaturation. In the ^{13}C-NMR spectral data of **2**, five carbon signals, δ_C 20.8, 78.9, 122.2, 129.2 and 170.4 were observed, indicating a symmetrical structure. In the ^{1}H-NMR spectral data of **2**, one methyl signal (H6), one methine signal (H5), and one olefinic proton signal (H4) were observed. The IR absorption band at 1755 cm^{-1}, the UV absorption band at 278 nm and one carbon signal (C2) indicated the presence of an α,β-unsaturated γ-lactone. The methyl group was linked to the oxygen-bearing methine proton ($J = 6.8$ Hz). The olefinic proton was adjacent to the methine group, as deduced from their coupling constant ($J = 2.0$ Hz). The IR absorption band at 3390 cm^{-1}, the positive reaction to *p*-dimethylaminobenzaldehyde and the remaining atoms from the molecular formula indicated the presence of one secondary amine. From these results, the planar structure of **2** was established to be di-3-(5-methyl-2,5-dihydro-2-oxofuryl) amine and the compound was named penidilamine. The ring-opening structure of the 3-(5-methyl-2,5-dihydro-2-oxofuryl) amino group appears similar to the structure of γ-hydroxynorvaline isolated from carpophores of *Boletus satanas)* (Matzinger, Catalfomo & Eugster, 1972). Interestingly, neither **1** nor **2** was obtained from cultures using potato extract medium.

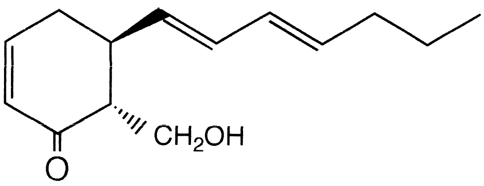
Compound **1** inhibited the hypocotyl elongation of lettuce seedlings by 45% at a concentration of 300 mg l^{-1}, but showed no inhibitory activity against root growth. The biological activity of **2** was not determined in this study due to its low yield (3 mg).



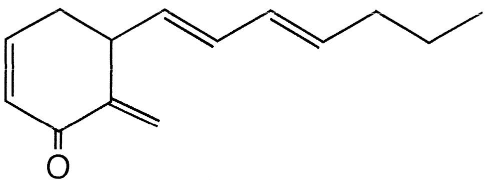
1: Peniamidienone



2: Penidilamine



3:Penienone



4:Penidienone

3. Experimental

3.1. General methods

General methods in these studies were as described previously (Kimura et al., 1998).

3.2. Isolation and purification of peniamidienone (*1*) and penidilamine (*2*)

Penicillium sp. No. 13, was cultured stationarily in Czapek–Dox medium at 24°C for 21 days. The culture broth (10 l) was filtered and the culture filtrate was adjusted to pH 2.0 with dilute HCl, then extracted twice with EtOAc. After evaporating the solvent, the residue (1.1 g) was purified by CC (C_6H_6–Me_2CO) on silica gel. The fr. (45 mg) eluting with C_6H_6–Me_2CO (99 : 1) was purified by prep. TLC developing with C_6H_6–Me_2CO (19 : 1) to afford 10 mg of **1** [R_f 0.67, C_6H_6–EtOAc (7 : 3)], as a yellow oil. The fr. (20 mg) eluting with C_6H_6–Me_2CO (49 : 1) was purified by prep. TLC on silica gel developing with C_6H_6–EtOAc (19 : 1) to afford 11 mg of penienone (**3**) [R_f 0.49, C_6H_6–EtOAc (7 : 3)]. Another fr. from this prep. TLC was recrystallized from *n*-hexane–Me_2CO to afford 3 mg of **2** [R_f 0.40, C_6H_6–EtOAc (7 : 3)], as a white powder.

3.3. Peniamidienone (1)

$[\alpha]_D^{25}$ −141° (MeOH; *c* 0.41); UV λ_{max}^{EtOH} nm (log ε): 204 (3.66), 227, (3.80), 260 (3.16), 370 (2.72); IR ν_{max}^{KBr} cm^{-1}: 2930, 1765, 1662, 1572, 1459, 1399, 1278, 1213, 1138, 991 and 829; HR-EIMS *m/z*: 313.1677 $[M]^+$ (*m/z* 313.1677 calculated. for $C_{19}H_{23}NO_3$); ^{1}H-NMR spectral data ($CDCl_3$): δ 0.95 (3H, *t*, *J* = 7.3 Hz, H13), 1.46 (2H, *m*, H12), 1.48 (3H, *d*, *J* = 6.8 Hz, H19), 2.09 (2H, *td*, *J* = 7.3, 6.8 Hz, H11), 2.36 (1H, *m*, H4), 2.55 (1H, *m*, H4), 3.38 (1H, *m*, H5), 5.10 (1H, *qd*, *J* = 6.8, 2.0 Hz, H18), 5.57 (1H, *dd*, *J* = 14.0, 7.8 Hz, H7), 5.68 (1H, *dt*, *J* = 14.2, 6.8 Hz, H10), 6.11 (2H, *m*, H8, 9), 6.20 (1H, *d*, *J* = 10.3 Hz, H2), 6.32 (1H, *d*, *J* = 2.0 Hz, H17), 6.77 (1H, *ddd*, *J* = 10.3, 4.4, 4.4 Hz, H3), 6.91 (1H, *dd*, *J* = 12.0, 0.5 Hz, H14), 11.0 (1H, *d*, *J* = 12.0 Hz, NH); ^{13}C-NMR spectral data ($CDCl_3$): δ 13.7 (*q*, C13), 20.2 (*q*, C19), 22.4, (*t*, C12), 31.8 (*t*, C4), 34.7 (*t*, C11), 41.0 (*d*, C5), 77.2 (*d*, C18), 110.7, (*s*, C6), 120.5 (*d*, C17), 129.6 (*d*, C9), 130.4 (*s*, C16), 131.2 (*d*, C7), 132.0 (*d*, C2), 132.6 (*d*, C8), 134.7 (*d*, C10), 139.0 (*d*, C14), 144.5 (*d*, C3), 168.5 (*s*, C15), 189.5 (*s*, C1).

3.4. Penidilamine (2)

Mp 187–189°C; $[\alpha]_D^{25}$ +33.0° (MeOH; *c* 0.31); UV λ_{max}^{EtOH} nm (log ε): 207 (3.83), 260 (3.50), 278 (3.64); IR ν_{max}^{KBr} cm^{-1}: 3390, 1755, 1676, 1537, 1337, 1153, 1115, 1084, 1024 and 810; HR-EIMS *m/z*: 209.0688 $[M]^+$ (*m/z* 209.0687 calculated. for $C_{10}H_{11}NO_4$); ^{1}H-NMR spectral data (Me_2CO-d_6): δ 1.39 (3H, *d*, *J* = 6.8 Hz, H6), 5.19 (1H, *qd*, *J* = 6.8, 2.0 Hz, H5), 6.65 (1H, *d*, *J* = 2.0 Hz, H4); ^{13}C-NMR spectral data (Me_2CO-d_6): δ 20.8 (*q*, C6), 78.9 (*d*, C5), 122.2 (*d*, C4), 129.2 (*s*, C3), 170.4 (*s*, C2).

3.5. Bioassay for the growth of lettuce seedlings

Bioassay using lettuce seedlings was described previously (Kimura et al., 1998; Frankland & Wareing, 1960).

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