Deoxycyclopaldic Acid and Cyclopaldic Acid, Plant Growth Regulators, Produced by *Penicillium* sp.

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Deoxycyclopaldic Acid, Cyclopaldic Acid, Growth Regulator

Deoxycyclopaldic acid (**1**) and cyclopaldic acid (**2**) were isolated from cultures of the fungus *Penicillium* sp. as plant growth regulators and their structures were established by spectroscopic evidence. Deoxycyclopaldic acid was found in nature for the first time. The biological activities of **1** and **2** have been examined using bioassay methods with lettuce and rice seedlings.

Introduction

We have investigated fungal metabolites as plant growth regulators, such as penienone [1, 2], penihydrone [1], penidienone [3], peniamidienone, penidilamine [4], and brevicompanines A and B [5]. In the course of our screening search for new plant growth regulators suitable for developing new herbicides and for new lead compounds, we found the presence of plant growth regulators in the cultural metabolites of *Penicillium* sp. Bioassay-guided fractionation led to isolation of the compounds deoxycyclopaldic acid (**1**) and cyclopaldic acid (**2**) [6–12]. In this report, we describe the structures and some biological activities of **1** and **2**.

Results and Discussion

Deoxycyclopaldic acid (**1**) and cyclopaldic acid (**2**) were isolated from the culture filtrate of a 21-day-old stationary culture of Penicillium sp. in a malt extract medium.

The molecular formula of **1** was determined by MS and elemental analysis to be $C_{11}H_{10}O_5$. The IR absorption band at 1757 cm^{-1} and one signal at δ = 167.3 in the ^{13}C NMR spectrum indicated the presence of an α,β-unsaturated γ-lactone. The ^{13}C and ^{1}H NMR spectra of **1** indicated the presence of a methyl, a methylene, a methoxy, a formyl, a hydrogen-bonded hydroxy and a hexa-substituted phenyl group. A NOE was observed between the formyl and the methylene protons. The hydroxy group was placed at C-5 because a hydrogen bond was formed between the hydroxy and the formyl groups. Two signals at δ = 163.5 and 166.8 in the ^{13}C NMR spectrum indicated that the hydroxy and the methoxy groups were in the *meta* relationship. From these results, **1** was identified as 4-formyl-5-hydroxy-7-methoxy-6-methylphthalide and the compound was named deoxycyclopaldic acid (Fig. 1). This compound had not been found before in nature and was only known as a synthetic product. The spectral data were identical to those reported for the synthetic product [7]. Compound **2** was identified as cyclopaldic acid (Fig. 1) by comparing the physicochemical properties with those reported [6, 7]. Cyclopaldic acid, which is produced by several species of *Penicillium, Aspergillus*, and *Pestalotiopsis*, shows antifungal activity, the phytotoxicity, and plant growth activity [6–12]. On the other hand, **1** shows antibacterial activity, but is not known as a plant growth regulator [8]. Furthermore, **1** and **2** are highly substituted phthalides and biological activities of these compounds are of interest because phthalide derivatives substituted at C-3 are rare as fungal metabolites [12, 13].

Plant growth activities of **1** and **2** toward lettuce and rice seedlings were examined. With lettuce seedlings (Fig. 2), **2** inhibited hypocotyl elongation and root growth of lettuce seedlings to 26% and 6% of control at a concentration of 100 mg/l, but **1** showed no inhibitory effect on the growth of lettuce seedlings at the same concentration. How-

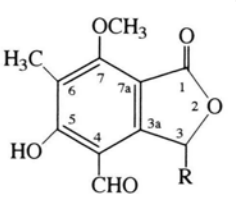

Fig. 1. Structures of deoxycyclopaldic acid (**1**) and cyclopaldic acid (**2**).

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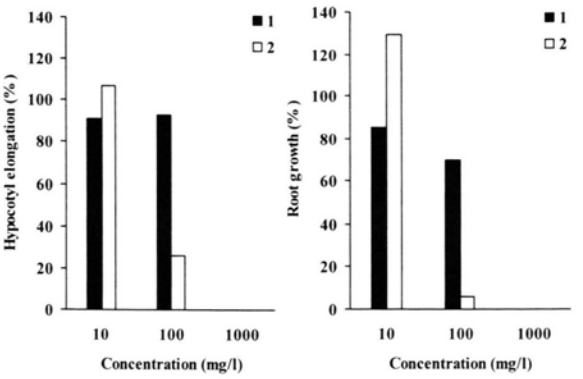

Fig. 2. Effects of deoxycyclopaldic acid (**1**) and cyclopaldic acid (**2**) on the growth of lettuce seedlings.

ever, **1** and **2** completely inhibited the growth of lettuce seedlings at a concentration of 1000 mg/l. On the other hand, **1** and **2** showed no inhibitory effect on the root and stem elongation of rice seedlings at a concentration of 100 mg/l (Fig. 3), but **1** completely inhibited the growth of the seedlings at a concentration of 1000 mg/l. However, **2** did not show any inhibitory effect on the stem elongation but inhibited the root growth to 23% of control at the same concentration. Cyclopaldic acid (**2**) in aprotic organic solvents exists as a lactone, but in aqueous solution, it is probably, in equilibrium with the aldehyde form, which may be considered the active molecule. Two aldehyde groups at C-3a and C-4 in **2** are essential for bioactivity [10, 11]. Deoxycyclopaldic acid (**1**), which does not have a masked aldehyde group at C-3a, showed less inhibitory activity than **2** in the bioassay method with lettuce seedlings but higher inhibitory activity than **2** toward rice seedlings. In particular, the difference in the inhibitory activity on the stem elongation is remarkable.

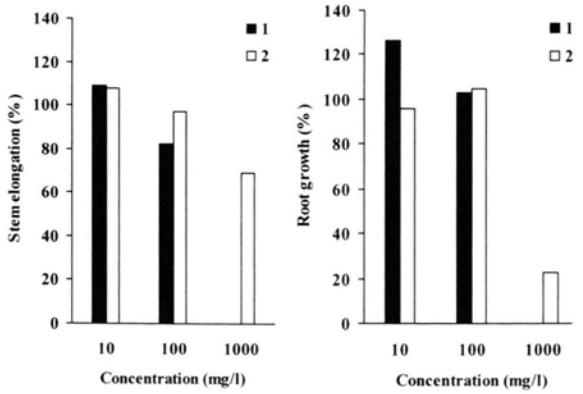

Fig. 3. Effects of deoxycyclopaldic acid (**1**) and cyclopaldic acid (**2**) on the growth of rice seedlings.

Experimental

UV and IR spectra were recorded on a Hitachi 100-50 and a JASCO FT/IR-7000 spectrometer, respectively. ^{1}H NMR and ^{13}C NMR spectra were obtained on a JEOL JNM GX-270 spectrometer. The MS spectrum was taken on a Hitachi RMU-6U spectrometer.

Isolation and purification of deoxycyclopaldic acid (**1**)

Penicillium sp. was cultured stationarily in a malt extract medium at 24 °C for 21 days. The culture broth (10 l) was filtered, and the filtrate was adjusted to pH 2.0 with 2 N HCl, before being extracted twice with EtOAc. The combined solvents were concentrated *in vacuo*, and the resulting residue was fractionated by column chromatography on silica gel (hexane–acetone). The active fraction eluted with 15% acetone was further purified by column chromatography on silica gel (hexane–acetone) and the solid was recrystallized from benzene–EtOAc to afford 129 mg of **1** (R_f: 0.42, benzene–EtOAc, 8:2, v/v) as colorless needles and 55 mg of **2** (R_f: 0.28) as colorless needles.

Physicochemical properties of deoxycyclopaldic acid (**1**)

M.p. 154–156 °C. – UV/vis (EtOH): λ_{max} (lg ε) = 258 (4.04), 295 (3.89), 365 nm (3.23). – IR (KBr): ν = 3390 (OH), 2932 (C=C), 1757 (O–C=O), 1652 (C=O), 1617 (C=C), 1583, 1490, 1457, 1401, 1350, 786 cm^{-1}. – ^{1}H NMR (270.05 MHz, $CDCl_3$): δ = 2.14 (s, 3H, Ar–*Me*), 4.19 (s, 3H, Ar–*O*Me), 5.50 (s, 2H, 3-H), 9.90 (s, ^{1}H, Ar–C*H*O), 12.05 (s, ^{1}H, O*H*). – ^{13}C{^{1}H} NMR (67.80 MHz, $CDCl_3$): δ = 7.9 (q, Ar–*Me*), 62.9 (q, Ar–O*Me*), 66.7 (t, C-3), 108.9 (s, C-7a), 110.3 (s, C-4), 119.9 (s, C-6), 152.3 (s, C-3a), 163.5 (s, C-7), 166.8 (s, C-5), 167.3 (s, C-1), 190.0 (d, Ar–*C*HO). – MS (EI): *m/z* (%) = 222 (73) [M$^+$], 204 (45), 193 (80), 176 (73), 164 (84), 77 (100). – $C_{11}H_{10}O_5$ (222.2): calcd. C 59.46, H 4.89; found C 59.38, H 4.68.

Bioassay for the growth of lettuce and rice seedlings

Bioassay methods with lettuce and rice seedlings were described previously [5].

[1] Y. Kimura, T. Mizuno, A. Shimada, Tetrahedron Lett. **38**, 469 (1997).
[2] Y. Kimura, T. Mizuno, T. Kawano, A. Shimada, Z. Naturforsch. **54b**, 1342 (1999).
[3] Y. Kimura, T. Mizuno, A. Shimada, T. Kawano, Phytochem. **47**, 323 (1998).
[4] Y. Kimura, T. Mizuno, T. Kawano, K. Okada, A. Shimada, Phytochem. **53**, 829 (2000).
[5] M. Kusano, G. Sotoma, H. Koshino, J. Uzawa, M. Chijimatsu, S. Fujioka, T. Kawano, Y. Kimura, J. Chem. Soc., Perkin Trans. 1, 2823 (1998).
[6] G. U. Brillinger, W. Heberle, B. Weber, H. Achenbach, Arch. Microbiol. **116**, 245 (1978).
[7] H. Achenbach, A. Muhlenfeld, B. Weber, W. Kohl, G. U. Brillinger, Z. Naturforsch. **37b**, 1091 (1982).
[8] H. Achenbach, A. Muhlenfeld, G. U. Brillinger, Liebigs Ann. Chem. 1596 (1985).
[9] A. J. Birch, M. Kocor, J. Chem. Soc. 866 (1960).
[10] L. Sparapano, A. Evidente, Nat. Toxins **3**, 156 (1995).
[11] G. D. Sorbo, A. Evidente, F. Scala, Nat. Toxins **2**, 136 (1994).
[12] W. B. Turner, D. C. Aldridge, in W. B. Turner, D. C. Aldridge (ed.): The Fungal Metabolites II, 68, Academic Press, London (1983).
[13] K. Kameda, M. Namiki, Chem. Lett. 1491 (1974).

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