Isolation of Aphidicolin as a Root Growth Inhibitor from *Harziella entomophilla*

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In the course of our screening research for new plant growth regulators among metabolites of microorganisms, we noticed the presence of a root growth inhibitor of lettuce and rice seedlings in the culture broth of *Harziella entomophilla* Ishiwata and Miyake. Then we attempted the isolation of the active principle and succeeded in the isolation of it as aphidicolin, I. In this paper we wish to report the isolation and identification of aphidicolin contained in the culture filtrate of the fungus as well as the biological activities of this compound.

The fungus was cultured stationarily in the Czapek-Dox medium containing 2.0% yeast extract for 25 days at 26°C. The culture broth thus obtained was filtered and the filtrate was extracted three times with ethyl acetate at pH 2.0. The combined extracts were washed with sodium bicarbonate solution and water, successively. After drying over anhydrous sodium sulfate, the extract was evaporated *in vacuo*. The residual syrup was applied onto a silica gel column (Wacogel C-200), which was eluted with benzene-ethyl acetate-methanol.

In the bioassay using lettuce and rice seedlings, marked inhibitory activity on the root growth was observed in an eluate with 100% ethyl acetate.

The crude solid, which was obtained on evaporation of the solvent, was recrystallized from ethyl acetate-methanol to give I as colorless needles melting at 242.0~242.5°C, in a yield of 8 mg from 1 liter of culture filtrate. After double development, I showed a single spot at R_f 0.48 and 0.38 on each plate (silica gel GF₂₅₄) developed with ethyl acetate-*n*-hexane-methanol (15:2:1, v/v) and benzene-acetone (1:1, v/v), respectively, and the spot was visualized by spraying with 50% sulfuric acid.

The molecular formula, C₂₀H₃₄O₄ was assigned to this compound through mass spectrometry [m/e : 307 (M⁺-CH₂OH), TMS derivative m/e : 626 (M⁺)] and elemental analysis (Anal. Found: C, 71.17; H, 10.03; O, 19.17. Calcd. for C₂₀H₃₄O₄: C, 70.97; H, 10.12;

O, 18.91%). $[\alpha]_D^{25} + 10.0$ ($c=2.1$, MeOH). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3520, 3440, 3360, 1077, 1047, 1027. PMR $\delta_{\text{TMS}}^{\text{Pyridine-d}_5}$ ppm: 0.76 (3H, s), 0.98 (3H, s), 1.2~3.0 (ca. 20H, br.m), 3.70 (5H, br.), 5.60 (1H, br.), 5.84 (1H, br.), 6.08 (1H, br.) CMR ppm (Pyridine-d₅): 15.4 (q), 18.0 (q), 23.5 (t), 25.4 (t), 27.2 (t), 27.3 (t), 27.3 (t), 29.0 (t), 31.7 (t), 33.3 (t), 34.0 (d), 40.1 (s), 40.5 (d), 40.9 (s), 42.0 (d), 49.6 (s), 63.3 (t), 71.8 (t), 74.2 (d), 76.3 (s).

On treatment with acetic anhydride in pyridine, I gave a triacetylated derivative (II) as crystals, followed by recrystallization from ethyl acetate-*n*-hexane. Mp 149.0~150.0°C. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3520, 1743, 1720, 1276, 1249, 1043. PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 1.00 (6H, s), 1.98 (6H, s), 2.06 (3H, s), 3.74 (1H, d, $J=8.0$ Hz), 3.98 (1H, d, $J=8.0$ Hz), 3.95 (2H, s), 4.80 (1H, m). MS m/e : 464 (M⁺), 391 (M⁺-CH₂OOCCH₃).

The bis-acetonide derivative III was prepared by treatment of I with dimethoxypropane and *p*-toluene sulfonic acid for 24 hr at room temperature. Physico-chemical properties of III recrystallized from methanol are as follows. C₂₀H₄₆O₄ (M⁺ 418). Mp 140.0~142.0°C. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 1204, 1099, 1060, 874. PMR $\delta_{\text{TMS}}^{\text{Pyridine-d}_5}$ ppm: 0.70 (3H, s), 0.96 (3H, s), 1.38 (6H, s), 1.20~2.32 (ca. 30H, br.), 3.19 (1H, d, $J=12.0$ Hz), 3.51 (1H, d, $J=8.0$ Hz), 3.52 (1H, m), 3.59 (1H, d, $J=12.0$ Hz), 3.71 (1H, d, $J=8.0$ Hz).

I; R¹=OH, R²=R³=CH₂OH
II; R¹=OAc, R²=R³=CH₂OAc

III

From these data mentioned above, I was seemed to be aphidicolin, an antimicrobial and antiviral tetrahydroxy diterpene from *Cephalosporium aphidicola* Petch¹⁾ and *Nigrospora sphaerica* (Sacc.).²⁾ Its identity was established by direct comparison of III with an authentic specimen of aphidicolin bis-acetonide³⁾ which was kindly sent by Dr. B. Hesp. The two were indistinguishable on a silica gel TLC and no depression in melting point was observed upon admixture. Moreover, physico-chemical data of III were identical with those of authentic bis-acetonide. Thus, I was determined to be aphidicolin.

I scarcely influenced the shoot growth of lettuce and rice seedlings. However, it markedly inhibited the root growth as shown in Fig. 1. At a dose of 20 mg/liter, the root growth of lettuce seedlings was suppressed to 50%. For the root growth of rice seedlings, I inhi-

FIG. 1. Effect of Aphidicolin on the Growth of Lettuce Seedlings.

Control length: 12.3 mm.

FIG. 2. Effect of Aphidicolin on the Growth of Rice Seedlings.

Control fresh weight: 11.6 g.

bited at a dose of 100 mg/liter to 50% and at a dose of 400 mg/liter the root growth was scarcely observed.

Further, biological activity of I was examined using *avena* coleoptile segments. I alone did not affect the straight growth of segments at dosages of 0.1~300 mg/

FIG. 3. Effect of Aphidicolin on the Growth of *Avena* Coleoptile Segments.

Control length: with IAA (0.3 mg/liter), 8.1 mm, ○---○; without IAA, 5.5 mm, ●—●.

liter. However, when applied with IAA at 0.3 mg/liter, I interfered the stimulative effect of IAA on the segments at a dose of 30 mg/liter.

Since aphidicolin is known as a potent inhibitor of cellular deoxyribonucleic acid synthesis of cultured human embryonic lung cells,⁴⁾ the root growth of lettuce and rice seedlings may be attributable to the inhibition of DNA synthesis.

This report seems to be the first one to show that aphidicolin possesses inhibitory activity on the plant growth.

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