Isolation of 2-Pyruvoylaminobenz-
amide as an Antiauxin from
Colletotrichum lagenarium

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In the course of our screening program to find substances with antiauxin activity among metabolites of phytopathogenic fungi, we noticed that a culture broth of *Colletotrichum lagenarium*, causative of anthracnose diseases in cucurbitaceous plants, counteracted the action of indole-3-acetic acid (IAA) applied to *Avena* coleoptile segments.

In this paper we wish to report the isolation and identification of 2-pyruvoylaminobenzamide (I) in the culture filtrate of the fungus as well as the biological activity of this compound.

Preliminarily, the fungus was aerobically cultured under shaking in a medium composed of 5% sucrose, 0.2% NaNO₃, 0.1% K₂HPO₄, 0.05% MgSO₄·7H₂O and 0.05% KCl for 7 days at 26.5°C. The culture broth was filtered and the filtrate was separated in the usual way by use of ethyl acetate into acidic, neutral and basic fractions, of which the neutral one revealed biological activity. Then, the active fraction was applied to thin-layer chromatography (silica gel GF₂₅₄; benzene-ethyl acetate, 1:1). The resulting chromatogram was divided into ten equal parts, which were extracted with acetone and bioassayed, respectively. The zone at *R_f* 0.3~0.4 showing biological activity was rechromatographed to give a crude product with a UV maximum at λ 211 nm (ϵ =16500).

For large scale preparation of this material, the fungus was cultured for 7 days at 26.5°C under aeration at a rate of 20 liter/min in a

20-liter jar containing 18 liters of Czapek-Dox medium (sucrose, 40 g/liter). In this case, the culture medium was not agitated with a mechanical stirrer, since the growth of the microbe was suppressed by stirring. Combined filtrates (80 liters) were acidified to pH 3.0 with 2 N HCl and extracted with ethyl acetate. The extract was concentrated to a small volume, followed by washing with satd. NaHCO₃ and water. After drying over anhydrous sodium sulfate, the extract was evaporated to dryness. The residual neutral oil (18 g) was subjected to silicic acid adsorption column chromatography (180 g, 2.7×55 cm). Elution was made successively with benzene and benzene-ethyl acetate increasing stepwise the content of the latter. Rechromatography of the active fraction eluted with benzene containing 30% ethyl acetate, followed by recrystallization from acetone gave colorless needles melting at 184~187°C (yield, 1.5 mg/liter).

Physico-chemical properties of the product are as follows.* C₁₀H₁₀N₂O₃. M⁺ 206.0690 (calcd. 206.0690). *P*K_a 10.28. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 211 (16500), 242 (9700) and 305 (6930). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3420, 3400~3200, 1730, 1690, 1660, 1620, 1590, 1520, 1360 and 755. NMR $\delta_{\text{TMS}}^{\text{DMSO}}$: 12.65 (1H, broad), 8.3 (1H, broad), 7.8 (1H, broad), 8.65 (1H, d, *J*=7.8 Hz) 7.92

FIG. 1. Synthetic Procedure of I.

* IR spectrum was run in Nujol mull on a JASCO IR-S spectrometer and NMR spectrum was recorded on a JEOL-JNM-4H spectrometer at 100 MHz. Mass spectrum was measured with a Hitachi RMU-6 at 70 eV and high resolution mass spectrum was obtained by a Hitachi RMH-2 with a direct inlet system at 70 eV.

FIG. 2. Effect of 2-Pyruvoylaminobenzamide (I) on the Growth of *Avena* Coleoptile Segments.

○---○ I, ●—● I+IAA (1 mg/liter).

(1H, d, $J=7.8$), 7.60 (1H, t, $J=7.8$), 7.24 (1H, t, $J=7.8$ Hz) and 2.50 (3H, s). MS m/e : 206 (M^+), 188 (M^+-188), 163 (M^+-43), 146, 119 ($C_7H_6NO^+$), 90 and 43 (CH_3CO^+). R_f value on TLC (silica gel GF₂₅₄; benzene-ethyl acetate, 1:1) is 0.31.

Chemical reactions of the compound including catalytic hydrogenation over PtO_2 , reduction with $LiAlH_4$ and hydrolyses with acid or alkali, as well as the data mentioned above suggested that the active compound must be 2-pyruvoylaminobenzamide, which had been isolated by Suter and Turner¹⁾ from *Penicillium chrysogenum* and *P. notatum*. The assumption unambiguously confirmed by synthesis using *o*-nitrobenzonitrile as a starting material (Fig. 1).

Biological activity of I was examined according to the method of Hirota *et al.*²⁾ by use

FIG. 3. Effect of 2-Pyruvoylaminobenzamide (I) on the Growth of *Avena* Coleoptile Segments.

○---○ IAA, ●—● IAA+I (10 mg/liter).

of *Avena* coleoptile segments. I at a concentration of 30 mg/liter did not affect the straight growth of segments (Fig. 2). However, when applied in combination with IAA at 1 mg/liter, I interfered with the response of segments to the hormone, even at a dose of 10 mg/liter. In this case, the inhibitory activity of I was reduced by increment of IAA applied simultaneously (Fig. 3).

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