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Isolation and Biological Activity of Pestalotin, a Gibberellin Synergist from *Pestalotia cryptomeriaeicola*

Sir:

During the course of our screening research for plant growth regulators with novel activity among microbial metabolites, we discovered a gibberellin synergist in a culture broth of *Pestalotia cryptomeriaeicola* Sawada, which is a fungus pathogenic for the Japanese cedar, *Cryptomeria japonica* D. Don. In this paper we wish to report the isolation and biological activity of this compound designated pestalotin.

The fungus was cultured under aeration 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 27°C. Acetone was added to the culture broth, which was then filtered and evaporated under reduced pressure. The residual solution was extracted with ethyl acetate at pH 3.0. After drying over anhydrous sodium sulfate, the extract was concentrated to a small volume and washed successively with a dilute sodium bicarbonate solution and water. After evaporation, the oily neutral fraction was subjected to silicic acid adsorption chromatography. Elution with benzene containing 30% ethyl acetate gave an active principle (yield, 3mg per liter culture broth). Recrystallization from *n*-hexane-benzene afforded pestalotin as colorless plates, mp 88.0~88.5°C. Its structure was assigned as I on the basis of spectral data.¹⁾

Growth-regulating activity of pestalotin on rice seedlings (*Oryza sativa* L., dwarf cultivar

'Kotake-tamanishiki') was examined according to the method of Murakami.²⁾ As illustrated in Fig. 1, pestalotin alone at dosages of 30~300mg/liter did not affect the growth of seedlings. However, when applied in combination with gibberellin A₃(GA₃), pestalotin

FIG. 1. Effect of Pestalotin on the Shoot Growth of Rice Seedlings (Kotake-tamanishiki) in the Presence or Absence of GA₃.

- GA₃(1.5mg/liter)+Pestalotin.
- GA₃(1.5mg/liter)
- Pestalotin
- Control

1) Y. Kimura, K. Katagiri and S. Tamura, *Tetrahedron Letters* (in press).

2) Y. Murakami, *Bot. Mag. Tokyo*, **72**, 36 (1959).

enhanced the stimulative effect of GA_3 on the shoot growth. Thus, pestalotin is probably the first compound whose synergetic action with gibberellin has been demonstrated.

It has well been known that gibberellins promote *de novo* synthesis of α -amylase in cereal grains.³⁾ Therefore, we examined in the following way whether or not pestalotin intensifies the effect of GA_3 on α -amylase induction in rice endosperms. Seeds (normal cutivar 'Tonewase') were dehusked and transversely cut in halves. The apical halves were soaked in 0.04% aqueous solution of methoxyethylenmercuric chloride for 30min, and then washed with sterile distilled water. A definite amount of pestalotin was dissolved in 1 ml of sterile distilled water in which varying amounts of GA_3 (0~3 μ g) had been

FIG. 2. Effect of Pestalotin on the Reducing Sugar Release in Embryoless Rice Endosperms in the Presence of Varying Amounts of GA_3 .

○—○ $GA_3 +$ Pestalotin (12.5mg/liter)
●—● GA_3

FIG. 3. Effect of Pestalotin on the Reducing Sugar Release in Embryoless Rice Endosperms in the Absence of GA_3 .

dissolved together with streptomycin sulfate (0.5 μ g). Ten endosperms were transferred into each solution in a test tube, which was incubated at 30°C for 40hr with intermittent shaking at every 12hr. Then the endosperms were removed and the total reducing sugar liberated into each solution was colorimetrically measured by the Somogy-Nelson method.

As shown in Fig. 2, the promotive effect of GA_3 on release of reducing sugar was enhanced by the simultaneous addition of pestalotin. Contrary to our expectations, pestalotin alone revealed capability to induce sugar release, though less active as compared with GA_3 as seen in Fig. 3.

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