Note

Isoxazole-4-carboxylic Acid as a Metabolite of *Streptomyces* sp. and Its Herbicidal Activity

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In the course of screening for herbicidal substances from microbes, we found that a culture filtrate of a strain of a soil actinomycete showed a marked phytotoxic effect against rice seedlings when applied on the submerged soil. An active principle was isolated in pure form and identified as isoxazole-4-carboxylic acid. The structure was finally confirmed by synthesis. This is the first report describing that isoxazole-4-carboxylic acid is a microbial metabolite and the compound has herbicidal activity.

The producing strain was isolated from a soil sample collected in Suwa, Nagano Prefecture. It grows well in organic media and produces abundant gray aerial mycelium and brown soluble pigment. It uses D-glucose but almost no other carbohydrates tested. Sporophores on yeast-starch medium are *Rectiflexibles*. Each spore chain contains more than fifty spores with smooth surface. Cell wall composition is L,L-diaminopimelic acid and belongs to type I. The menaquinone composition is MK-9 (H₆). From these observations, it is clear that the strain belongs to the genus *Streptomyces* and it is referred to as *Streptomyces* sp. RK-489. The species was not identified.

Fermentation was done in a jar fermentor containing 18 l of the medium consisted of 2% glucose, 1% soluble starch, 0.1% meat extract, 0.4% dry yeast, 2.5% soy bean meal, and 0.2% NaCl. The seed culture (140 ml) incubated for 2 days at 27°C in flasks was used for inoculation. Jar fermentation was done for 120 hr at 27°C under aeration of 10 l/min and agitation of 200 rpm.

The fermentation broth was filtered and the filtrate was passed through a column of Amberlite IRA-45 (OH⁻ form, 5 l). After washing with water, an active principle was eluted by 1 N NaCl. The eluate was passed through a column of active charcoal, which was washed with water and then eluted with 10 to 30% aqueous acetone. The eluate was concentrated *in vacuo* and neutralized with 0.1 N

NaOH, then put onto a column of Sephadex G-10. After development with water, active fractions were combined and concentrated *in vacuo* to a small volume and lyophilized. The residue was washed with a mixture of acetone–hexane, affording 100 mg of a white amorphous powder of the sodium salt.

The compound is soluble in water and lower alcohols and does not melt below 250°C. From the elementary analysis of the sodium salt (Found: C, 37.34; H, 1.79; N, 11.67%. Calcd. for C₄H₂NO₃Na: C, 35.55; H, 1.48; N, 10.37%) and HR-EI-MS of the free acid (M⁺ 113.0114. Calcd. for C₄H₃NO₃: 113.0114), the molecular formula of the sodium salt was established as C₄H₂NO₃Na.

It has a UV absorption maximum at 215 nm in water ($E_{1\text{cm}}^{1\%}$ 277) and reduced permanganate. It showed a couple of singlets (δ_{H} 8.45, 8.84 ppm) in ¹H-NMR spectrum in DMSO-*d*₆ and four signals (δ_{C} 122.5, 151.0, 161.2, and 168.2 ppm) in the ¹³C NMR spectrum in CD₃OD. Its IR spectrum in KBr indicated the presence of carboxylate (1580 and 1400 cm⁻¹) (Fig. 1). From the data described above, the structure, isoxazole-4-carboxylic acid, was deduced.

The proposed structure was confirmed by the synthesis, which was accomplished by the condensation of ethyl 2,2'-diformylacetate and hydroxylamine followed by acid hydrolysis as described by Panizzi.^{1,2} Comparison with the natural product was done by IR (Fig. 1), ¹H and ¹³C NMR spectra.

The compound showed phytotoxicity against rice seedlings when applied on the submerged soil at the rate of a.i. 7.5–15 g/10a. The seedlings were grown to the 1.5–2 leaf stage with Kumiai Ryujobaido K in plastic pots (diameter 6 cm and volume 180 ml) with drainage holes in an air-conditioned green-house, then 40 ml of 0.5–1.0 µg/ml

Fig. 1. IR Spectra of Isoxazole-4-carboxylic Acid Sodium Salt (in KBr).

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of aqueous solution of the compound was poured on the pots covered with paper cups (diameter 7 cm and volume 205 ml), and herbicidal activity was evaluated after 5 days. Herbicidal effect against various plants is under investigation. The compound showed no antimicrobial activity against bacteria, fungi, or yeasts tested and 200 mg/kg of oral administration to mice caused no toxic sign.

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References

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