Note

Isolation of Acetylaranotin,
Bisdethiodi(methylthio)-
acetylaranotin and Terrein as
Plant Growth Inhibitors
from a Strain of
Aspergillus terreus

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In the course of our research project¹⁾ on plant growth regulators among fungal metabolites we noticed that an *Aspergillus terreus* strain, C-520, which was isolated from a soil sample collected in Takarazuka city, produced several plant growth regulators. One of them is a plant growth promoter and its structure elucidation is in progress. Three of them are plant growth inhibitors. In this paper we wish to report the isolation, identification and biological activities of these three plant growth inhibitors produced by *Aspergillus terreus* C-520.

Asp. terreus C-520 was cultured in Czapek-Dox medium (40 liters) fortified with yeast extract on a shaking machine for three days at 30°C. The culture filtrate was extracted with ethyl acetate and the neutral extract was concentrated *in vacuo*. The concentrate was subjected to silicagel column chromatography (Wako gel C-200). Elution was performed successively with chloroform and chloroform-acetone, increasing stepwisely the content of the latter. The eluate with chloroform and that with chloroform-acetone (80:20) showed inhibition of growth of lettuce seedlings (*Lactuca sativa* L., Great Lakes 366). A growth promoter for lettuce seedlings was contained in the eluate with chloroform-acetone (90:10). The eluate with chloroform was further chromatographed on a silicagel column with a solvent system of *n*-hexane and ethyl acetate (80:20), and then a Sephadex LH-20 column with methanol. Two biologically active substances designated as C-520A (I) and C-520B (II) were isolated as yellow crystals (30 mg) and a yellow powder (5 mg), respectively. The eluate with chloroform-acetone (80:20) was rechromatographed on a silicagel column with a solvent system of *n*-hexane-ethyl acetate. Crude crystals of an active substance were obtained from the eluate with *n*-hexane-ethyl acetate (60:40). Recrystallization from acetone-ethyl acetate gave colorless needles of C-520D (III) (200 mg).

I decomposed above 200°C on a hot stage. IR $\nu_{\text{max}}^{\text{KBr}}$ 1740, 1670, 1230 cm^{-1} . The molecular formula of I was determined to be $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_8\text{S}_2$ from elemental analysis and the high resolution mass spectrum of I. Anal. Found: C 53.86; H 5.06; N 5.49; S 11.78. Calcd. for $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_8\text{S}_2$: C 53.93; H 4.86; N 5.24; S 11.78%. HR-MS: M^+ m/z 534.1172 (Calcd. for $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_8\text{S}_2$ 534.1130); $\text{M}^+ - \text{SCH}_3$ m/z 487.1221 (Calcd. for $\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_8\text{S}$ 487.1175). I was positive to chlorine-*o*-toluidine-KI and palladium chloride²⁾ and decolorized aqueous potassium permanganate solution. $^1\text{H-NMR}$ (60 MHz, CDCl_3 , TMS): δ 2.05 (6H, s), 2.2 (6H, s), 3.0 (4H, m), 4.6 (2H, dd), 5.75 (2H, m), 6.25 (2H, dd), 6.5 (2H, m). I was deduced to be a symmetric diketopiperazine. In conclusion, these data mentioned above coincide with those for bisdethiodi(methylthio)-acetylaranotin,³⁾ which is a member of the aranotin group.

II decomposed above 200°C on a hot stage. IR $\nu_{\text{max}}^{\text{KBr}}$ 1730, 1690, 1220 cm^{-1} . Since II gave the same color reaction as I and the IR spectrum of II was very similar to that of I, II was presumed to belong to the aranotin group. In the mass spectrum of II the molecular ion peak was not observed and the base peak was at m/z 440. The composition of the base peak as judged by the high resolution mass spectrum was $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_8$ (Found m/z 440.1188. Calcd. for $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_8$ 440.1218). By comparison of the behavior on thin layer chromatography⁴⁾ and the mass spectra of I and II, II was determined to be acetylaranotin.³⁾ Acetylaranotin had already been isolated as a biologically active substance against rhino-, Coxsackie, polio- and parainfluenza viruses,⁵⁾ while bisdethiodi(methylthio)acetylaranotin had no antiviral activity.⁵⁾

III melted at 127°C and its molecular formula was found to be $\text{C}_8\text{H}_{10}\text{O}_3$ from elemental analysis and the high resolution mass spectrum. Anal. Found: C 62.10; H 6.63%. Calcd. for $\text{C}_8\text{H}_{10}\text{O}_3$: C 62.34; H 6.49%. HR-MS: M^+ m/z 154.0588 (Calcd. for $\text{C}_8\text{H}_{10}\text{O}_3$ 154.0628). IR $\nu_{\text{max}}^{\text{KBr}}$ 3350, 1675, 1620, 1210 cm^{-1} . III was positive to 2,4-dinitrophen-

FIG. 1. Structures of I, II and III.

ylhydrazine and decolorized aqueous potassium permanganate solution. $^1\text{H-NMR}$ (60 MHz, $(\text{CD}_3)_2\text{CO}$, TMS): δ 2.0 (3H, d, $J=6$ Hz), 3.0 (1H, s), 4.1 (1H, d), 4.7 (1H, d), 4.9 (1H, br. s), 5.9 (1H, s), 6.3 (1H, d, $J=18$ Hz), 6.8 (1H, dq, $J=18$ and 6 Hz). The signals at δ 3.0 and 4.9 disappeared on addition of D_2O . These data coincide with those for terrein.⁶⁾

The structures of **I**, **II** and **III** are shown in Fig. 1.

The bioassay was conducted at 25°C under continuous light (ca. 2000 lux) with lettuce seedlings for three days and rice seedlings (*Oryza sativa* L., cv. Koshihikari) for seven days. **I** reduced root elongation of lettuce seedlings by about 40% at a concentration of 100 ppm, but **II** reduced it by about 80% even at 10 ppm. **III** reduced the root length of lettuce seedlings to 20% at 50 ppm. However, the inhibitory effect of **I**, **II** and **III** on the hypocotyl growth was not clearly observed at these concentrations. **I** did not affect growth of rice seedlings even at 100 ppm, while **II** reduced the total root length by 50% at 50 ppm and by 80% at 100 ppm. A dose of 100 ppm of **III** showed 80% inhibition of the root elongation of rice seedlings and 40% inhibition on the total length (length of second leaf sheath plus length of second leaf).

II remarkably inhibits growth of lettuce and rice seedlings. On the other hand, the inhibitory activity of **I** is comparatively low.⁷⁾ The disulfide bridge seems to be necessary for higher biological activity.

Though the yield of **III** by *Asp. terreus* C-520 is low, *Asp. fischeri* Wehmer was reported to produce **III** in a high yield (5 mg/ml).⁸⁾ There is a possibility to get derivatives of **III** with higher activity by structural modification.

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