Altenin, a New Phytopathologically-toxic Metabolite from *Alternaria Kikuchiana*

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Alternaria Kikuchiana Tanaka is a fungus which produces black spots on leaves and fruits of the pear, especially the improved species *Pyrus Ussuriensis var. Aromatica* Rehder (Japanese name *Nijusseiki*).[2] This fungus had been cultivated on a Czapek-Dox medium containing 4 per cent sucrose.[3] For the large-scale preparation it has now been cultivated in a 200-l. fermenter on a sucrose, ammonium phosphate, potassium phosphate and potassium chloride medium for 8 days at 30°C.

A factor which developed black spots on the leaf of the pear was isolated from the culture filtrate; this was designated "Altenin." Only one drop of an aqueous 2×10^{-5} mg./ml. solution of altenin exhibited the phytopathologic effect. Thin-layer chromatography indicated that no other active substance was present in this filtrate. After the adjustment of the culture filtrate to pH 3.0, the active substance was adsorved on active charcoal and then eluted with acetone. The acetone was evaporated, and the altenin was purified by passing it through columns of silica gel and alumina.

One milligram of altenin was obtained from about 4 l. of the culture filtrate. Altenin is a yellow liquid, giving only one spot on silica-gel thin-layer chromatography with a benzene-acetone (1 : 1 v/v) mixture; the R_f value is 0.76, while that of methyl red, a pilot dye, is 0.37. On alumina thin-layer chromatography with a methanol-water (5 : 1 v/v) mixture, the R_f value is 0.87, while that of methyl red is 0.41.

Altenine is unstable at high temperatures and loses its biological activity in 10 min. at 80°C, or in an hour at 60°C. At 40°C, however, the activity continues for several hours, while at room temperature it is stable for 2 days, even when a stream of air is blown into the aqueous solution.

A molecular formula of $C_9H_{14}O_6$, (M. W. 218) was indicated by elementary analysis and mass spectrometry. Altenin is soluble in water, alcohol, ethyl ether, acetone and chloroform, and moderately soluble in benzene. It is extracted from acidic solutions with ethyl ether, but it is not extracted from alkaline solutions. A pK_a value of 7.75 is indicated by potentiometry. The infrared absorption spectrum shows OH and carbonyl bands, but no carboxylic acid bands. The nuclear magnetic resonance spectrum shows a multiplet equivalent to two hydrogens, which disappears upon deuterium exchange. Altenin has a levo specific rotation at the D line.

Structural studies of altenin are now in progress; the details will be reported in the near furture.

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2) I. Hiroe, S. Nishimura and W. Sato, *Transactions of the Tottori Society of Agriculture Science (Tottori Nogei Kagaku Kai Shi)*, **11**, 291 (1958).

3) R. Mohri and S. Ikeda, *The Liberal Art Journal of Natural Science of Tottori University (Tottori Daigaku Gakugei Gakubu Kenkyu Hokoku)*, **10**, 38 (1959).