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A Flycidal Constituent of *Amanita pantherina* (DC.) Fr.

Amanita pantherine (DC.) Fr. is a poisonous fungus containing muscarine and choline as poisonous constituents. This fungus, as well as *Amanita muscaria* (L.) Pers. and *Tricholoma muscarium*, has long been known to be flycidal. We now describe isolation of a flycidal constituent from *Amanita pantherina* (DC.) Fr., which is water-soluble and supposed to be an amino acid or a peptide, since it was adsorbed on Amberlite IR-120 (H-type) and IRA-410 (OH-type).

The fresh fungus was extracted with 50% ethanol and the extract was passed through an IR-120 column, which was then eluted with 4% aquous ammonia. The eluate was concentrated, passed through an Amberlite IRC-50 (H-type) column and rinsed first with water to give the fraction-I (acidic to neutral), and then with 10% aquous pyridine (fraction-II) and finally with 4% aquous ammonia (fraction-III). Of these, the fraction-II was most active, followed by the fraction-I, while the fraction-III was inactive.

The fraction-II contained about 10 components, of which histidine formed a main constituent, as could be revealed by paper chromatography. Histidine was then removed as sparingly soluble flavianate and the remaining portion was adsorbed on IR-120, followed by elution with ammonia. The eluate was evaporated and the residue was dissolved in 60% ethanol and filtered through a silica gel column to give a number of fractions. Each fraction was tested for its activity and the active fraction were combined, evaporated and the residue was again dissolved in 60% ethanol and treated similarly with an alumina column. The purification method was repeated for the third time through a silica gel column and the final product was purified from 80% ethanol to give slightly colored prisms melting at 174~176° (decomp.). *Anal.* Found : C, 42.39; H, 5.35; N, 23.26. PPC*1 : Rf 0.44 (BuOH-AcOH-H_2O=3:1:1), Rf 0.61 (phenol-H_2O=4:1). TLC*2: Rf 0.46 (BuOH-AcOH-H_2O=3:1:1). Reaction with ninhydrin gave orange coloration which turned violet after a few hours' standing. Infrared spectra : see Fig. 1.

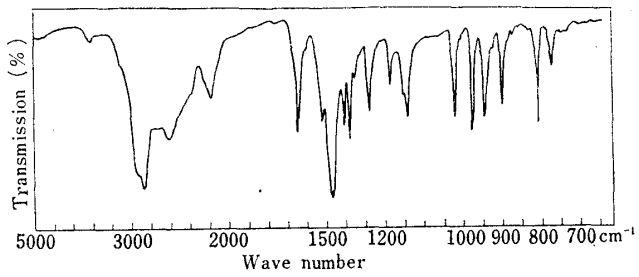

Fig. 1. Infrared Spectrum of Pantherine in Nujol

The hydrolyzed product, obtained by heating the above mentioned substance at 120° in 6*N* hydrochloric acid in a sealed tube for 48 hours, was no longer active and 3 amino acids were revealed by thin-layer chromatography,*2 Rf 0.22, yellow; 0.28, pink; 0.40, orange (BuOH-AcOH-H_2O=3:1:1).

Hence the original compound is a peptide and we propose to call it pantherine.

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*1 Toyo Roshi No. 51.
*2 Silica gel G, 0.25 cm.