

STUDIES ON THE TOXINS OF *PYRICULARIA*

2. Detection of Phriculol in Blast Diseased Leaves of Gramineae

TAMARI AND KAJI considered α -picolinic acid and pyricularin as the toxins of *Pyricularia oryzae* and detected both compounds in extracts from diseased rice plants⁷. However, all attempts in this laboratory to identify these two toxins in cultures of *Pyricularia* from rice or other Gramineae and in diseased leaves of their respective hosts have not been successful. Recently, other phytotoxic substances, viz., pyriculol², 3, 4-dihydro-3, 4, 8-tri-hydroxy-1(2H)-naphthalenone¹ and tenuazonic acid¹ have been isolated from cultures of the rice blast fungus. Of these, tenuazonic acid has also been isolated from blast-diseased rice plants⁸. Although pyriculol has been shown to cause a dark necrotic spot, resembling the natural blast lesion, on rice leaves and also inhibit growth of rice seedlings^{5,6}, this compound does not appear to have been detected in blast-diseased leaves of rice or other gramineous hosts. It has been reported that pyriculol could be detected not only in cultures of *P. oryzae* but also in cultures of *Pyricularia* from other cultivated and wild Gramineae⁴. It was, therefore, of interest to study if pyriculol could be detected in blast-diseased leaves of various Gramineae.

Six gramineous hosts, viz., *Oryza sativa* (CO 13), *Setaria italica* (CO 1), *Eleusine coracana* (CO 5), *Panicum repens*, *Brachiaria mutica* and *Leersia hexandra* were raised in a temperature controlled growth room³. The six hosts were inoculated with conidial suspensions of the respective isolates of *Pyricularia*. Lesions were collected on the fourth day of inoculation when the symptoms were discernible by their water-soaked appearance.

One gram each of the lesions was extracted with 80% methanol in a mortar using acid washed sand. The extracts were centrifuged at 7,000 r.p.m. for 15 min and the methanol in the supernatant driven off *in vacuo* at 50° C. The aqueous residue was re-centrifuged and the supernatant extracted with 3 volumes of ethyl acetate. The ethyl acetate extracts were pooled and taken to dryness *in vacuo* at 50° C. The residue was dissolved in 1.0 ml of ethyl acetate and 0.5 ml of the sample was streaked on a 250 μ thick silica gel (without binder) thin-layer plate previously activated at 120° C for 30 min. Authentic pyriculol was used as the marker. The plate was first developed with benzene and subsequently with benzene : ethyl acetate (9 : 1, 8 : 2 and 7 : 3 v/v) solvent systems to a distance of 100 mm. Authentic pyriculol could be located at an R_f of 0.39 under U.V. (356 nm) after the final development. The marker and the region cor-

responding to the marker were scrapped off, eluted with ethanol and scanned in the U.V. region for their absorption spectra in a UNICAM SP 800 Spectrophotometer. The spectra of the samples were compared with that of authentic pyriculol.

Of the extracts from the six gramineous hosts studied, pyriculol was detectable only in the case of blast lesions from *B. mutica* (Fig. 1) although

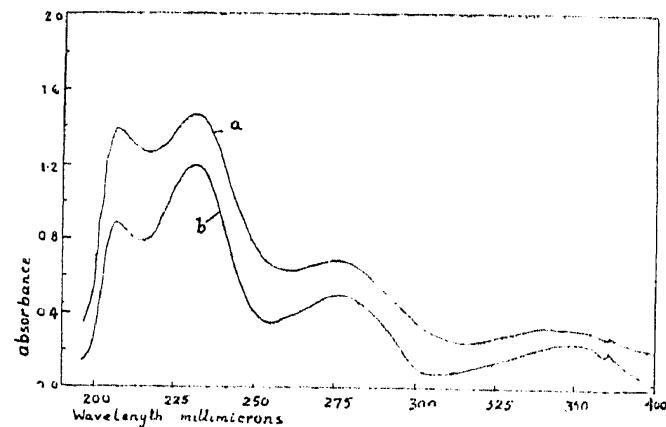


FIG. 1. U.V. spectra of pyriculol detected in blast lesions from *B. mutica* (a) and authentic pyriculol (b) in ethanol.

the compound was detectable in culture filtrates of all the isolates used here except the one from rice⁴. The amount of pyriculol in blast lesions from *B. mutica* was estimated from the O.D. value at 232 nm with reference to known standards prepared from authentic pyriculol and this amounted to 132 μ g/g fresh weight of lesions. Although pyriculol could not be detected in blast lesions from the other hosts, its detection *in vivo* reported here and that it can be frequently isolated from cultures of *Pyricularia* from many gramineous hosts⁴ suggest a possible role for this polyketide in the blast disease syndrome.

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