

Degradation of Pisatin by Fungi of the Genus *Fusarium*

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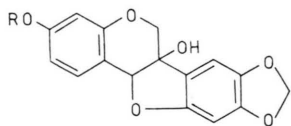
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Fifteen strains of *Fusarium* previously shown to degrade flavonoids and isoflavonoids were investigated for pisatin degradation. *Fusarium anguioides* and *Fusarium avenaceum* converted the phytoalexin (**1**) to the nontoxic 3,6a-dihydroxy-8,9-methylenedioxypterocarpan (**2**).

Increasing evidence in recent years [1–3] points to the ability of various fungi to degrade fungitoxic phytoalexins to less toxic compounds. Such degradative reactions are of interest in view of our understanding of host-parasite relationship, because phytoalexin degradation has been visualized as an ability of pathogenic fungi to overcome a chemical barrier directed by the plant against further spread of infection [4].



1. R = CH₃ 2. R = H

Recent studies [5, 6] on polyphenol degradation by 15 selected strains of the genus *Fusarium* demonstrated the pronounced capability of these fungi for the catabolism of a large variety of aromatic structures. The observation of strong O-demethylation reactions from the 7 position of isoflavones [6] has led to similar studies on the degradation of the phytoalexin pisatin (**1**) because removal of O-methyl groups has been postulated as introductory step in the fungal metabolism of certain pterocarpan phytoalexins [1, 7].

Pisatin (**1**) isolated from infected pea seedlings and purified according to [7] was incubated (10⁻⁴ M) with suitable amounts of fungal mycelium from our *Fusarium* strains [6]. Pisatin metabolism was followed in aliquots by UV-spectroscopy and TLC. 13 of the 15 strains showed no indication

of pisatin metabolism within 48 hours though all strains well degrade isoflavones [6]. Most of these strains seemed to be severely effected by the phytoalexin because in the presence of pisatin a steep decrease in the excretion of various coloured and fluorescing compounds from the cells into the medium could be observed. Strain VII (*Fusarium anguioides* Sherbakoff, CBS 172.323) and strain VIII (*Fusarium avenaceum* (Fr.) Sacc., CBS 386.623), however, quantitatively converted **1** accumulating a red-violet fluorescing compound **2** which migrates much slower on TLC (S2 *R_F* = 0.1; S4 *R_F* = 0.22; S5 *R_F* = 0.16) than **1**. The UV-spectrum of (**2**) (max 281, 286 and 309 nm) is indistinguishable from that of **1**, does not change upon addition of boric acid though it is subject to a bathochromic shift upon addition of base. Unlike pisatin (**2**) reacts strongly with diazotised *p*-nitroaniline to give an orange product.

Methylation of **2** with diazomethane afforded a compound with the same UV spectrum and TLC mobility (S2 *R_F* = 0.44; S3 *R_F* = 0.28; S5 *R_F* = 0.57) as pisatin. Strains VII and VIII therefore O-demethylated pisatin (**1**) to 3,6a-dihydroxy-8,9-methylenedioxypterocarpan (**2**) possessing a much lower antifungal activity [7]. *Stemphylium botryosum*, *Fusarium solani* f. sp. *Pisi* and *Ascochyta pisi* have previously been shown [8–10] to also initiate pisatin metabolism by O-demethylation at C-3 (pterocarpan numbering). This appears to exemplify a process of generalized microbial metabolism of isoflavonoids [1, 6, 11]. The restricted number of pisatin-degrading strains of *Fusarium* in comparison to those degrading isoflavones [6] demonstrates that phytoalexin degradation may be a rather specific property. The data suggest that screening of pathogenic strains of *Fusarium* for pisatin degradation may represent a means to correlate different degrees of pathogenicity with the capability of phytoalexin degradation.

Experimental

Fungi. Cultivation, growth and pretreatment of fungi for experiments were as previously described [6].

Chromatography. TLC on silica gel was performed with solvent systems S1: CHCl₃:CH₃OH 25:1; S2: C₆H₆:EtOAc:iso-PrOH 90:10:1; S3: toluene:EtOAc 7:1; SA: C₆H₆:EtOAc:iso-PrOH 90:20:2; S5: CHCl₃:CH₃OH 97:3.

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Pisatin. The phytoalexin was obtained from fungi-infected pea seedlings (*Pisum sativum* L. "Nebelungs Imperiala") according to established methods [7] and finally purified in systems S1, S2 and S3. Care was taken to avoid acidic dehydration of **1** (pH >6) and photodestruction by wrapping all flasks with aluminium foil.

Incubations. All degradative studies were carried out as previously described. **1** was added in minimum amounts of 2-methoxyethanol. Aliquots from control flasks without substrate were used as UV reference.

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