Degradation of Pisatin by Fungi of the Genus Fusarium

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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].

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 introductionary 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} \text{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-violett fluorescing compound 2 which migrates much slower on TLC (S2 RF = 0.1; S4 RF = 0.22; S5 RF = 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 (1) reacts strongly with diazotized p-nitroaniline to give an orange product.

Methylation of 2 with diazomethane afforded a compound with the same UV spectrum and TLC mobility (S2 RF = 0.44; S3 RF = 0.28; S5 RF = 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. Pisii and Ascochyta pisii 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: CHCl_3 : CH_3OH 25 : 1; S2: C_6H_6 : EtOAc : iso-PrOH 90 : 10 : 1; S3: toluene: EtOAc 7 : 1: SA: C_6H_6 : EtOAc : iso-PrOH 90 : 20 : 2; S5: CHCl_3 : CH_3OH 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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