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DETOXIFICATION OF PHASEOLLIDIN BY *FUSARIUM SOLANI* f.sp. *PHASEOLI*

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Abstract—The phytoalexin phaseollidin is transformed into phaseollidin hydrate by liquid mycelial cultures and cell-free culture filtrates of *Fusarium solani* f.sp. *phaseoli*. The antifungal activity of the hydrate is much less than that of the original phytoalexin.

INTRODUCTION

The resistance of plant tissues towards fungal attack may be due, at least in part, to the accumulation of phytoalexins [1]. Diseased tissue of French bean (*Phaseolus vulgaris*) frequently contains several phytoalexins, including phaseollin, kievitone, phaseollinisoflavan and phaseollidin (**1**) [1, 2]. *Fusarium solani* (Mart.) Sacc. f.sp. *phaseoli* (Burk.) Snyd. et Hans, a bean pathogen involved in root and foot rot [3], has the capacity to metabolize phaseollin to 1α-hydroxyphaseollone [4, 5] and kievitone to kievitone hydrate, the latter through the activity of kievitone hydratase [6–8]. Both metabolites are less inhibitory to fungal growth than the parent compounds and it has been suggested that such detoxification processes could contribute to pathogenesis. [8]. Phaseollinisoflavan also disappears from cultures of *F. solani* [9] and phaseollidin appears to be transformed by the enzyme kievitone hydratase [8]; the products of these reactions were not characterized.

The present paper confirms the metabolism of phaseollidin by *F. solani*, establishes the structure of the metabolite formed and provides details of its antifungal activity.

RESULTS

Characterization of phaseollidin hydrate

When phaseollidin (20 μg/ml) was added to liquid mycelial cultures of *F. solani*, a metabolite was detected which migrated at a lower R_f (0.20) than phaseollidin (0.36) on Si gel TLC. Both compounds, however, produced identical orange–brown reaction products upon treatment with diazotized *p*-nitroaniline. The UV spectrum of the metabolite (λ_{max}^{EtOH} 282 and 287.5 nm) was also similar to that of phaseollidin [10]. Within 3–5 hr, phaseollidin could not be detected in the cultures, whilst the amount of metabolite present increased to a maximum (8 μg/ml) during this period and this amount was maintained for at least a further 20 hr.

The same metabolite was formed in liquid cultures pretreated with small amounts of kievitone (5–10 μg/ml) for 6–16 hr before addition of phaseollidin. Likewise, cell-free filtrates from kievitone-induced cultures gave rise to the same product after addition of phaseollidin. The metabolite was only detected in cultures or filtrates to which phaseollidin had been added.

Analysis of the metabolite by MS showed M^+ 342.2 (13%), with two fragment ions at *m/e* 324.3 (39%) and 268.1 (100%). These ions were clearly derived by successive loss of H_2O and isobutene. The ^1H NMR spectrum revealed two major differences when compared with that of phaseollidin [11]. The olefinic proton resonance at δ 5.28 of phaseollidin was absent from the metabolite and the non-equivalent methyls of phaseollidin at δ 1.67 (3H) and 1.76 (3H) were replaced by a sharp singlet at δ 1.22 (6H). This demonstrated that a hydrate had been formed by a specific Markonikov addition of H_2O across the double bond of the isopentenyl group yielding the tertiary alcohol. Anti-Markonikov addition would have produced a secondary alcohol in which the methyl groups would have been split into a doublet by the adjacent proton. The structure of the metabolite is therefore **2** and, as suggested earlier [8], will be referred to as phaseollidin hydrate.

Antifungal activity of phaseollidin hydrate

A TLC assay using *Cladosporium cucumerinum* indicated that the lowest detectable amounts of phaseollidin and phaseollidin hydrate were 0.5 and 5 μg, respectively. Further studies confirmed that the hydrate is much less inhibitory than phaseollidin. For example, although phaseollidin appreciably reduced both radial mycelial growth on agar and increases in mycelial dry wt

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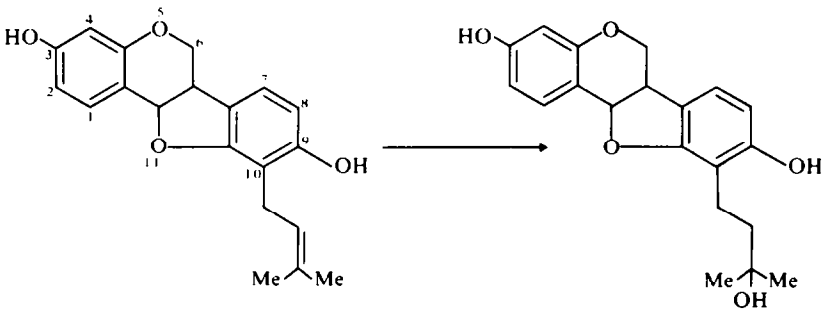

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in liquid medium, similar concentrations of the hydrate had little effect on fungal growth (Table 1). The exception to these effects was the ability of *F. solani* to grow on an agar medium containing phaseollidin. This result is, however, consistent with previous data [9] and is proabably due to detoxification during the assay period (see also [12, 13]).

More detailed studies on fungal cells showed that, although phaseollidin was completely fungicidal at concentrations of 10 or 20 μg/ml, the hydrate exhibited little inhibitory activity at these concentrations (Table 2). Even at 100 μg phaseollidin hydrate per ml, 'growth' was rarely reduced by more than 50%, although assessments of hyphal viability showed that some sporelings were killed.

DISCUSSION

Fusarium solani f.sp. *phaseoli* is an aggressive pathogen in French bean hypocotyls [9]. It has already been suggested that this pathogenic capability may, to some extent, be due to its ability to detoxify the host's phytoalexins [8]. Characterization of phaseollidin hydrate and the demonstration of its reduced antifungal activity add further support to this proposal.

Hydration of the isopentenyl group caused a large reduction in antifungal activity. However, this change did

Table 1. Comparative antifungal activities of phaseollidin and phaseollidin hydrate

	Fusarium solani f.s p. *phaseoli*		*Aphanomyces euteiches*		*Rhizoctonia solani*	
	Growth (mm)	Dry wt (mg)	Growth (mm)	Dry wt (mg)	Growth (mm)	Dry wt (mg)
Control	23.7	33.9	19.2	7.9	16.2	7.5
Phaseollidin	20.3 (85)	6.6 (19)	8.3 (43)	4.7 (59)	5.9 (36)	2.4 (32)
Phaseollidin hydrate	23.0 (97)	30.9 (91)	17.6 (92)	7.0 (88)	15.4 (95)	6.9 (92)

Growth on agar and dry wt in liquid culture were determined by procedures reported elsewhere [16]. Concentrations employed were 100, 20 and 50 μg/ml agar and 50, 10 and 25 μg/ml liquid medium for *F. solani*, *A. euteiches* and *R. solani*, respectively. Growth was measured after 28 hr (*A. euteiches* and *R. solani*) or 5 days (*F. solani*). Each figure is the mean of three replicates.

Table 2. Effects of phaseollidin and phaseollidin hydrate on *Colletotrichum lindemuthianum*

Assay	0*	Inhibition (% of control) 5	10	20	50	100
Spore germination on agar						
Phaseollidin	0	31	99	100	100	100
Phaseollidin hydrate	0	−1	1	2	17	47
Spore germination in liquid medium						
Phaseollidin	0	26	100	100	100	100
Phaseollidin hydrate	0	0	−2	4	12	42
Sporeling growth on agar						
Phaseollidin	0	40	35	100 (100)†	100 (100)	100 (100)
Phaseollidin hydrate	0	7	5	5	28	66
Sporeling growth in liquid medium						
Phaseollidin	0 (0)	80 (64)	100 (98)	100 (100)	100 (100)	100 (100)
Phaseollidin hydrate	0 (0)	3 (0)	33 (0)	24 (10)	7 (14)	29 (40)

Assays were carried out using sucrose–casein hydrolysate medium [13]. Results, 50 measurements in each of two replicates, were taken after incubation for 24 hr.

*Concn in μg/ml.

†Values in parentheses represent % sporelings killed.

not appear to affect the mode of action since the hydrate, like phaseollidin, was able to kill fungal cells, although only at higher concentrations.

Since cell-free filtrates from cultures which had received small amounts of kievitone could also transform phaseollidin, it seems likely that the same enzyme, kievitone hydratase [8], is responsible for the transformation of both phaseollidin and kievitone. It will, therefore, be of interest to determine whether other isoflavonoids containing isopentenyl groups, e.g. licoisoflavone A [14] or weighteone [15], would also form hydrates in the presence of this enzyme.

EXPERIMENTAL

Phaseollidin was isolated from tobacco necrosis virus-infected hypocotyls of *P. vulgaris* cv Prince and purified [10]. Mycelial cultures of *F. solani* f.sp. *phaseoli* were obtained as described elsewhere [8]. Phaseollidin and kievitone were dissolved in EtOH and added to cultures such that the final EtOH concn was between 0.5 and 1%. Cell-free culture filtrates were prepared as outlined previously [8]. After incubation at 25°, the cultures or culture filtrates were extracted [7]. Phaseollidin hydrate was purified by TLC on Si gel G developed in hexane–Me_2CO (2:1) and on Si gel 60, F254 (Merck 5715) using EtOH–$CHCl_3$ (3:97). Quantification of the hydrate was based on the UV λ_{max}^{EtOH} (log ε) 287.5 nm (3.80) of phaseollidin [10].

Radial mycelial growth and liquid culture (dry wt) bioassays using *Aphanomyces euteiches* Drechs, *Rhizoctonia solani* Kühn and *F. solani* were carried out as described in ref. [15] and measurements of the germination of spores and growth of 1-day-old sporelings of *Colletotrichum lindemuthianum* race γ as in ref. [12]. Viability of fungal cells was assessed using fluorescein diacetate [17] and 8-anilino-1-naphthalene sulphonic acid [18].

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