

### Postinfectional Inhibitors from Plants. III. Detoxification of Capsidiol, an Antifungal Compound from Peppers

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#### ABSTRACT

Capsidiol, an antifungal sesquiterpene induced in pepper fruit by several fungi is oxidized to the ketone, capsenone, by *Botrytis cinerea* and *Fusarium oxysporum* f. *vasinfectum*. Capsenone is less fungitoxic than capsidiol,

and its formation may be a detoxification mechanism of importance in overcoming host resistance.

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It was reported previously that an antifungal sesquiterpene (capsidiol, Fig. 1-a) is induced in pepper fruit (*Capsicum frutescens* L.) by a number of fungi. Roughly, the concentrations of capsidiol found in the diffusates are inversely related to the pathogenicity of the fungi tested, in keeping with a possible role of the compound in the natural defense of the plant (4). An explanation of how such concentration differences arise is therefore of significance.

We report now that diffusates from the interaction of pepper fruit (Keystone Resistant Giant) with *Botrytis cinerea* Pers. ( $3 \times 10^6$  spores in 6 ml injected into the cavity of each fruit) or *Fusarium oxysporum* Schlecht. f. *vasinfectum* (Atkinson) Snyder ( $4-7 \times 10^6$  spores in 6-10 ml similarly injected) contain a second compound easily revealed by thin-layer

chromatography. Under comparable conditions, this compound was not detected in noninoculated pepper fruit nor in interactions with any of the other six fungi previously tested. The compound was isolated by ether extraction, purified by preparative thin-layer chromatography [silica gel, Camag DF5; *sec*-butanol-ethyl acetate-acetic acid (5:95:0.1, v/v);  $R_F$  ca. 0.54; strong fluorescence quenching], and proved identical in every respect with capsenone (Fig. 1-b) previously obtained in the laboratory by chromic acid oxidation of capsidiol (M. Gordon, A. Stoessl, & J. B. Stothers, unpublished data). Typical yields of capsenone were: *F. oxysporum* f. *vasinfectum*, 31 mg from 285 ml diffusate from 105 fruit; *B. cinerea*, 17.4 mg from 260 ml diffusate from 66 fruit. These are comparable to the yields of capsidiol obtained previously in diffusates from fruit injected with *Monilinia fructicola*. The extracts also contained small quantities of capsidiol (1-2 mg).

Evidence was obtained that capsenone is readily formed from added capsidiol by pure cultures of both *B. cinerea* and *F. oxysporum* f. *vasinfectum*. The fungi were grown at 25 C in shake culture in a synthetic medium (2.36 g L-asparagine, 15 g D-glucose, 1 g  $\text{KH}_2\text{PO}_4$ , 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 100  $\mu\text{g}$  thiamine-HCl, 100  $\mu\text{g}$  pyridoxine-HCl, trace amounts of salts of Fe, Zn, Co, Mn, Cu, Ca, N KOH to adjust the pH to 6, 1,000 ml  $\text{H}_2\text{O}$ , dispensed in 50-ml aliquots in 200-ml Erlenmeyer flasks) supplemented after 36 hr with either 1 ml of  $5 \times 10^{-3}$  M capsidiol in ethanol or with 1 ml ethanol as control. After a further 48 hr, most of the capsidiol had been destroyed, and capsenone was isolated from the cultures of both fungi and identified as above. In contrast, in similar experiments with *M. fructicola* and *Phytophthora capsici* the capsidiol concentration was unchanged and no trace of capsenone could be detected.

The comparative fungitoxicity of capsidiol and capsenone was determined by standard spore germination assays (4) with *B. cinerea*, *F. oxysporum* f. *vasinfectum*, and *M. fructicola* (Table 1). It is evident that the fungi are considerably less sensitive to capsenone, and that the conversion could be an effective detoxification mechanism.

It must be concluded, therefore, that although biosynthesis of capsidiol in peppers is stimulated by

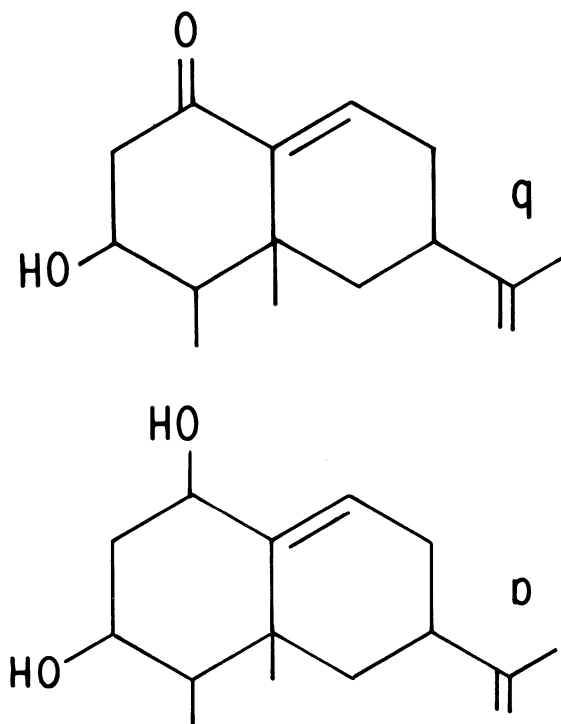


Fig. 1. a = Capsidiol; b = capsenone.

TABLE 1. Inhibition of spore germination of *Botrytis cinerea*, *Fusarium oxysporum* f. *vasinfectum*, and *Monilinia fructicola* by capsidiol and capsenone

		% Inhibition			
		$10 \times 10^{-4}$ M <sup>a</sup>	$7.5 \times 10^{-4}$ M	$5 \times 10^{-4}$ M	$2.5 \times 10^{-4}$ M
<i>B. cinerea</i>	Capsidiol	100	31	0	0
	Capsenone	26	17	0	0
<i>F. oxysporum</i> f. <i>vasinfectum</i>	Capsidiol	100	95	56	13
	Capsenone	50	16	0	0
<i>M. fructicola</i>	Capsidiol	100	100	55	26
	Capsenone	22	0	0	0

<sup>a</sup>Concentration of inhibitory compounds.

several fungi, the low amounts previously found after injection with spore suspensions of *B. cinerea* or *F. oxysporum* f. *vasinfectum* are probably due to its conversion to capsenone and not to a lack of induction potential in these two fungi. Culture filtrates from *F. oxysporum* also induce capsidiol and not capsenone; this will be reported in detail in a subsequent communication. As may be the case with some pterocarpanoid phytoalexins (1, 2, 3, 5), any function capsidiol may have in the defense of the plant may be circumvented by these pathogens by means of its destruction. Obviously the low amounts of capsidiol in fruit injected with *P. capsici*, recorded previously (4), are not due to this mechanism. In this case the fungus, which is highly pathogenic, either has a lower capacity for induction or more probably, in some manner inhibits the induction process.

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