Postinfectional Inhibitors from Plants. VI. Capsidiol Production in Pepper Fruit Infected With Bacteria

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ABSTRACT

Capsidiol, the antifungal sesquiterpene induced in fruit of pepper (*Capsicum frutescens* L.) by fungi was demonstrated in bacterial soft rot lesions in fruit collected from the field. In laboratory experiments, *Erwinia carotovora* isolated from rotted fruit induced small quantities in diffusates and larger amounts in rotted tissue. Capsidiol (1 μmole/ml) was not active against the bacteria isolated and hence is not of significance in resistance to bacterial soft rot.

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Previous reports have described laboratory experiments on the induction and breakdown by fungi of capsidiol, the antifungal sesquiterpene from pepper fruit (6, 7, 8). To determine if capsidiol accumulates in naturally diseased pepper fruit, soft rot infected fruit were collected in the field and examined for the presence of capsidiol. Also, the ability of soft rot bacteria was compared with the ability of *Monilinia fructicola* (Wint.) Honey to stimulate the accumulation of capsidiol in ripening pepper fruit.

Pepper fruit, (*Capsicum frutescens* L., 'California Wonder') with conspicuous bacterial soft rot, and visually without evidence of infection of other kinds, were collected from the field. After removal of healthy tissue the rotted portions were weighed and extracted first by steeping overnight in sufficient diethyl ether to submerge the tissue and then, after submerging the residue in water, twice more with roughly half-volumes of ether. The combined extracts from 1.88 kg diseased tissue (wet wt) were dried with sodium sulphate and yielded, on evaporation, 1.39 g of a red syrup. This was chromatographed on a column of 100 g aluminum oxide (Woelm, neutral grade III; column 18 mm i.d.). Fractions, 50 ml each, were eluted with chloroform

(fractions 1-12) and 2% methanol in chloroform (fractions 13-24). Fractions 17-21 gave crude crystalline capsidiol (201 mg). This was recrystallized from ether after treatment with charcoal to furnish pure capsidiol (93 mg, 0.21 μmoles/g fresh weight), melting point and mixture melting point 150-152 C. Identity was further confirmed by the nuclear magnetic resonance spectrum. In 60 g of apparently healthy tissue collected at the same time only trace amounts of capsidiol were detected. In another experiment a similarly rotted sample of tissue (44 g) was carefully extracted with ether and the dried extract analyzed by gas-liquid chromatography (GLC), as previously described (7). The capsidiol concentration was 0.52 μmoles/g fresh weight. These levels are of the same order as those obtained from diffusates from peppers in response to *Monilinia fructicola* and other fungi (6).

Bacterial isolates from field-infected fruit were tentatively identified as *Erwinia carotovora* (L. R. Jones) Holland by Dr. J. W. Rouatt, Chemistry and Biology Researchg Institute, Ottawa, Canada. Ability to induce capsidiol in pepper fruit was tested using methods described previously for fungi (6). Five ripening fruit ('Keystone Resistant Giant', greenhouse-grown) of roughly equal size, were each injected with 5 ml of a suspension of bacterial cells (10^{10} cells/ml in sterile saline, from loop-inoculated shake cultures, grown on nutrient broth for 18 hr at 27 C). For controls, fruit were similarly injected with a spore suspension of *Monilinia fructicola* (5 ml per fruit, 5×10^5 spores/ml) or with sterile saline. After incubation at room temperature for 44 hr, the fruit were opened and the diffusates collected and extracted with ether. Capsidiol levels in the extracts were determined by GLC. The amount of capsidiol induced by the bacteria (0.04 μmoles/ml diffusate) was small compared to that induced by *M. fructicola* (0.41 μmoles/ml diffusate) but much more than in the saline control (trace, < 0.001 μmoles/ml diffusate).

It was found that whole fruit injected as above rotted very slowly, and sometimes not at all, over a period of 2-3 wk while sliced tissue was macerated in a few days. Furthermore, green tissue rotted much more rapidly than ripened tissue, a situation apparently similar to that in tomatoes recently discussed by Bartz and Crill (1). Further evidence of the production of capsidiol in inoculated tissue was obtained as follows. Green fruit, calyx removed, were washed in detergent solution, thoroughly rinsed with water, surface-sterilized for 5 min with 0.5% hypochlorite, rinsed with sterile distilled water and sliced (2-3-mm thick) under aseptic conditions. The slices were transferred to preweighed, capped, sterilized flasks, and sprinkled with a bacterial suspension (10^{10} cells/ml, 10 ml/flask, approximately 60 g tissue). The flasks were shaken to thoroughly distribute the suspension and incubated at 27 C for 6 days. Despite these precautions we were not successful in completely eliminating bacteria from control tissue slices and some rotting occurred there also. The rotted tissue was extracted and analyzed by GLC as above. Yields of capsidiol were 0.068 μmoles/g fresh weight inoculated tissue and 0.012 μmoles/g fresh weight uninoculated tissue.

Assays of capsidiol against the bacterial isolates [cup-plate method and turbidometric assay (4)] were negative up to 1 μmole/ml, the highest concentration tested.

Other reports of the induction by bacteria of substances considered to be phytoalexins are those for phaseollin in beans (2, 5) and rishitin and phytuberin in potatoes (3). Like capsidiol, phaseollin was not active against the inducing bacteria and neither compound can be assumed to have any importance in resistance to bacterial disease. The induction of capsidiol by bacteria provides a further example of the non-specific nature of such processes.

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