Accumulation of Wyerone in Broadbean and Demethylhomopterocarpin in Jack Bean after Inoculation with Phytophthora megasperma var. sojae

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ABSTRACT

Extracts from broadbean and jack bean hypocotyls inoculated with the nonpathogen, *Phytophthora megasperma* var. *sojae*, contained antifungal compounds that were not detected in extracts from noninoculated plants. The induced antifungal compounds have been identified as wyerone from broadbean and demethylhomopterocarpin from jack bean.

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Additional key words: phytoalexins, polyacetylenes, pterocarpans.

Smith (8) reported that jack bean (*Canavalia ensiformis* [L.] DC.) plants produced an antifungal substance after fungus inoculation. Fawcett et al. (3) isolated and elucidated the structure of the performed antifungal compound wyerone from broadbean (*Vicia faba* L.) plants and later (2) showed that this compound increased after fungus inoculation. I report data demonstrating that demethylhomopterocarpin (medicarpin) and wyerone are produced by jack bean and broadbean hypocotyls, respectively, after inocuation with *Phytophthora megasperma* Drechs. var. *sojae* A. A. Hildb.

Plants were grown from seed in growth chambers as previously described (5). Broadbean (cultivar Long Pod) seed was purchased from Burpee Seed Co., Riverside, Calif. Seed of an unnamed jack bean variety was generously supplied by J. S. Kirby, Department of Agronomy, Oklahoma State University, Stillwater. Six- to 10-day-old plants were inoculated in hypocotyl wounds with mycelial pieces of the nonpathogen *Phytophthora megasperma* var. *sojae* as previously described (5), and incubated in a moist chamber for 48-72 hr. The hypocotyls were then harvested and extracted with 95% ethanol and ethyl acetate (5). The crude ethyl acetate extracts were chromatographed on 0.375-mm layers of Silica Gel GF-254 (Brinkman Insts., New York, N.Y.) with various solvent systems. Thin-layer chromatography (TLC) plates were viewed under ultraviolet light and bioassayed for antifungal compounds with *Cladosporium cucumerinum* spores (5). Antifungal compounds were recovered from the TLC plates by elution of the silica gel with acetone. Spectral analyses were made of antifungal compounds which appeared to be pure in a number of TLC solvent systems.

Antifungal activity was not detected by the TLC bioassay in extracts of wounded, noninoculated plants, but extracts from inoculated jack bean and broadbean hypocotyls contained single antifungal compounds.

The antifungal compound from jack bean was isolated from hypocotyls 48 hr after inoculation by preparative TLC with hexane-ethyl acetate-methanol (60/40/1, v/v) followed by benzene-methanol (98/2, v/v). It could be located on the plates by its absorbance when the plates were illuminated with light having peak emission at 254 nm. The isolated antifungal substance gave an ultraviolet spectrum indistinguishable from homopterocarpin [λ_{max} = 279(s) and 284 nm]; however, the presence of a free hydroxyl group was indicated by the infrared spectrum and an observed base shift in the ultraviolet spectrum. The mass spectrum (M^+ = 270; prominent fragments at m/e 255, 239, 237, 227, 226, 209, 197, 181, 161, 148, and 147) was indistinguishable from that of an authentic sample of demethylhomopterocarpin (medicarpin) obtained from Verna Higgins, University of Toronto, Canada, thus establishing the identity of this compound. Milligram quantities of demethylhomopterocarpin were easily obtained by extracting cotyledons which had been sliced in half longitudinally and mycelium of *P. megasperma* var. *sojae* and water placed on the wounded surfaces for 48-96 hr in petri dish moist chambers. Fifty cotyledons yielded from 4 to 12 mg of purified demethylhomopterocarpin in three experiments.

The broadbean antifungal compound was isolated by preparative TLC with pentane-diethyl ether-acetic acid (75/25/1, v/v) followed by a second chromatography with benzene/isopropanol (95/5, v/v) to remove sterol contaminants. The compound was located on the plates by its dark blue fluorescence under ultraviolet light. The purified antifungal compound yielded the following spectral data: the mass spectrum gave M^+ = 258 with major fragments at m/e 227, 226, 199, 179, 151; diagnostic infrared bands were observed at 4.6, 5.9, 6.2, 6.8, and 6.95 μ; λ_{max}^{ETOH} = 351 nm; NMR (ppm in $CDCl_3$): 1.1 (triplet), 2.4 (doublet-quartet), 3.8 (singlet), 5.7 (doublet), 6.4 (doublet), 6.7 (doublet), 7.3 (doublet), 7.7 (doublet). The similarity of these data to those of wyerone (3) established the identity of this compound.

Based on ultraviolet spectral and solubility data, demethylhomopterocarpin was probably the induced antifungal compound observed previously in diffusates from jack bean pods by Smith (8). Demethylhomopterocarpin has previously been identified as an inducibly produced antifungal compound in alfalfa (7) and red clover (4). Occurrence of the same compound in jack beans represents the first reported production of an induced antifungal pterocarpan by two different Tribes of the *Leguminosae* subfamily Lotoideae.

Although wyerone is a preformed compound in broadbean (2, 3), I did not detect it in extracts from noninoculated plants with the TLC bioassay. Wyerone was the only antifungal compound observed in extracts from broadbean hypocotyls inoculated with *P. megasperma* var. *sojae*. It also increased markedly in broadbean inoculated with *Botrytis fabae* (2). I did

not detect wyerone acid, previously isolated from *Botrytis*-infected plants (6), or viciatin (1), a presumed pterocarpanoid compound for which a structure has not been offered.

Data are not available to assess critically whether production of these antifungal compounds and resistance of jack bean and broadbean to the nonpathogen *P. megasperma* var. *sojae* are causally linked.

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