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XANTHOTOXIN: A PHYTOALEXIN OF *PASTINACA SATIVA* ROOT*

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XANTHOTOXIN was isolated from the top mm of parsnip root discs inoculated with the fungus *Ceratocystis fimbriata*, a nonpathogen of parsnip. The compound was characterized by TLC, GLC, UV, NMR, IR, MS and comparison to authentic xanthotoxin. The content of xanthotoxin in the top mm of inoculated tissue and control tissue treated with water, 72 hr after inoculation or treatment, was *ca.* 1·0 mg and 0·05 mg/g fr. wt, respectively. Inoculation with the nonpathogens of parsnip *Helminthosporium carbonum*, *Alternaria* sp. or *Colletotrichum lindemuthianum* β race also resulted in accumulation of xanthotoxin. A concentration of 1×10^{-3} M xanthotoxin prevented growth of *C. fimbriata* and 1×10^{-4} M inhibited radial growth of the fungus *ca.* 50%.

EXPERIMENTAL

Parsnip roots were washed with detergent and water, rinsed with deionized water, and surface sterilized with 95% EtOH for 3–5 min. The roots were cut into 8–10 mm thick slices under aseptic conditions and transferred into sterile glass Petri dishes. The upper surfaces of the slices were inoculated with conidial suspension of *Ceratocystis fimbriata* (*ca.* 4×10^6 conidia/ml) or covered with sterile water (control). The plates were held in the dark at 60% humidity and 25° for 72 hr. After incubation, control tissue showed little change in appearance, whereas the inoculated tissue was extensively browned in the top mm and some browning was evident to the second mm from the surface. Tissue below the second mm appeared normal. The top mm of tissue was removed, weighed, boiled in 95% EtOH for 5 min, and homogenized for 3 min. The homogenate was filtered, the residue washed with 95% EtOH, and the filtrate and washings were evaporated to dryness. The residue was dissolved in 95% EtOH to give a final concentration equivalent to 1 g fr. wt of tissue/ml. 50 μl of the concentrate were streaked on silica gel G plates and developed in cyclohexane–ethyl acetate (1:1). One yellow (R_f 0·67, compound C) and two blue-white (R_f 0·34, compound A, and R_f 0·47, compound B) fluorescing bands were visible in uv light (366 nm) on plates streaked with extract from inoculated tissue. The bands were barely discernible on plates streaked with extracts of control tissue. The three bands were clearly visible on plates streaked with extracts of tissue inoculated with *H. carbonum*, *Alternaria* sp. or *C. lindemuthianum* β-race, but the amount of compounds A–C was less than that on plates streaked with extracts of tissue inoculated with *C. fimbriata*. TLC plates sprayed with a spore suspension of *H. carbonum* in Czapek's liquid medium indicated that the three bands were fungitoxic. The major inhibitory band, corresponding to Compound C, was eluted with 95% EtOH, applied to antibiotic discs (equivalent of 0·1 g fr. tissue/disc), and the discs were applied to potato dextrose agar inoculated with spores

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of *C. fimbriata*. Zones of inhibition were clearly evident around discs containing C. In subsequent experiments, C was incorporated into potato dextrose agar and radial growth of the fungus was determined. The equivalent of 10^{-3} M xanthotoxin, allowed little or no growth of the fungus. Inhibition of fungal growth was *ca.* 50% with concentrations of C, equivalent to 1×10^{-4} M xanthotoxin.

For purification, the dry residue from the crude extract was dissolved in a minimal amount of EtOAc. Separation was achieved on a silica gel column with cyclohexane–EtOAc using successively higher concentrations of EtOAc: 2, 5, 10, 20, 30, 50 and 100%. Ten ml fractions were collected and examined by TLC. Fractions with only C visible under UV were combined and submitted for analyses.

Elemental analyses of purified C Found: C, 66·71; H, 3·83; O, 28·69. Calc. for $C_{12}H_8O_4$: C, 66·67; H, 3·70; 0, 29·63%). The UV, IR, NMR and MS patterns for C and authentic xanthotoxin were identical. The latter technique yielded a high resolution parent ion of 216 for C (MW xanthotoxin, 216·18). The corrected m.p. of C, crystalized from 80% EtOH, and xanthotoxin were 146–147° and 145–147°, respectively.

For GLC studies, purified xanthotoxin, under the name of methoxsalen, was obtained from Upjohn, Inc. A 4 ft 3% OV1 chromasorb W 80/100 mesh vapor phase chromatography column with a hydrogen flame detector was used at 175° to measure the concentration of xanthotoxin and to confirm its identity. GLC of the inoculated crude extract indicated three major peaks with R_ts of 0·6, 1·6 and 4·8 min. GLC of xanthotoxin indicated only one peak at 4·8 min. GLC of control extract had two trace peaks with retention times identical to the first and third peaks of the crude extract. As a final test, xanthotoxin was mixed with the inoculated extract. The resulting GLC pattern gave the same three peaks as before. However, the area under the third peak was greatly enlarged. GLC was also used to quantitate xanthotoxin in extracts of control and inoculated tissue.

Parsnips are known to contain at least six coumarins: angelicin, psoralin, isopimpinellin, bergapten, xanthotoxin, bergapten and imperatorin.[1–3] Under certain stress situations, at least one of these compounds, xanthotoxin, has been found to increase in parsnip tissue. The accumulation of xanthotoxin in parsnips is analagous to the accumulation of 6-methoxymellen in carrot root.[4] Whether xanthotoxin acts like a phytoalexin in the other plants in which it is normally found in trace amounts remains to be determined.

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