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CAMALEXIN ACCUMULATION IN *ARABIS LYRATA*

MICHAEL ZOOK, LISSA LEEGE,† DAVID JACOBSON‡ and RAY HAMMERSCHMIDT*

Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, U.S.A.; †Department of Plant Biology, The Ohio State University, Columbus, OH, U.S.A.; ‡Department of Plant Biology, Carnegie Institute of Washington, 290 Panama Street, Sanford, CA 94305, U.S.A.

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Key Word Index—*Arabis lyrata*; Brassicaceae; biosynthesis; phytoalexins; camalexin.

Abstract—Inoculation of leaves of *Arabis lyrata* with either a bacterial pathogen *Pseudomonas syringae* pv. *maculicola* strain ES4326 or *Cochliobolus carbonum*, a fungal nonpathogen of *A. lyrata*, resulted in the accumulation of a compound with similar chromatographic and fluorescent properties to that of camalexin (**I**), a phytoalexin produced by *Arabidopsis thaliana*. *A. lyrata* is closely related to *A. thaliana*. High resolution electron impact mass spectroscopic and proton NMR analysis confirmed that the compound produced by *A. lyrata* is camalexin.

INTRODUCTION

Phytoalexin production by plants represents one of the major defense responses studied in plant–pathogen interactions [1]. Within the last ten years, a number of phytoalexins have been characterized from the Brassicaceae, a family that represents a number of economically important plant species. Within this plant family, all of the phytoalexins characterized are indole derivatives with a sulfur and nitrogen containing moiety attached at the 3 position of the indole ring [2].

The genus *Arabis* consists of approximately 120 species of small mustards that are distributed across the Northern hemisphere [3]. In the last several years, interest in the ecology of Arabis has increased [4–7]. Several studies have focused on the infection of natural populations of *A. holboelli* by the rust fungi *Puccinia monoica* and *P. thalspeos* and *A. lyrata* by the white rust pathogen *Albugo candida* (Refs. [8, 9] and Jacobson, unpublished observations).

Although disease has been studied in natural populations of plants, all of these studies have concentrated on the population genetics of resistance rather than mechanisms [10]. The *Arabis* relative *Arabidopsis* has become a model plant for studies on host resistance and a number of genes for resistance and putative resistance mechanisms have been identified. Because *A. lyrata* and *A. thaliana* are closely related [11, 12] it is likely that *A. lyrata* may serve as a good model plant to study mechanisms of disease resistance in natural plant populations by using defense and resistance genes cloned from *Arabidopsis*. A number of genes for resistance have been characterized in *A. thaliana* and the nature of host resistance responses have been studied. Thus, much of what has been learned in *A. thaliana* host–parasite interactions may be directly applicable to *A. lyrata*.

As an initial step in understanding how *A. lyrata* resists infection, we investigated the possibility that *Arabis* produces phytoalexins. In this paper we report on the induction and chemical characterization of a phytoalexin from *A. lyrata*.

RESULTS AND DISCUSSION

Inoculation of leaves of *A. lyrata* with either *P. syringae* pv. *maculicola*, a bacteria that causes bacterial leaf spot disease of members of the plant family Brassicaceae [13, 14], or *C. carbonum*, a fungal nonpathogen of *A. lyrata*, resulted in the accumulation of a compound with similar properties to that of camalexin (**I**) (Fig. 1), a phytoalexin produced by *Arabidopsis thaliana* [15] and *Camalina sativa* [16]. The *A. lyrata* compound had the same R_f of 0.6 as camalexin on silica gel G60 TLC plates developed with $CHCl_3$:MeOH (9:1, v/v) and the same bluish-purple fluorescence under short-wave UV light.

*Author to whom correspondence should be addressed.

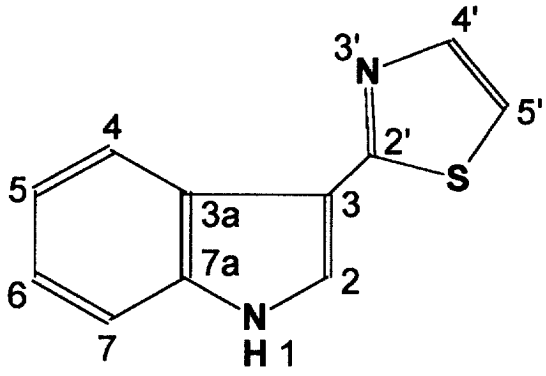

Fig. 1. Structure of camalexin (I) (3-thiazol-2'-yl-indole).

Electron impact mass spectroscopic analysis of the *A. lyrata* compound generated major mass fragments of 142 and 58. These mass fragments are the same as those reported for camalexin isolated from *C. sativa* [16] and *A. thaliana* [15]. High resolution mass spectroscopic analysis of the molecular ion of the *A. lyrata* compound was 200.0410. The same measurement of the molecular ion of camalexin from *C. sativa* and *A. thaliana* was 200.0403 and 200.0401, respectively. The theoretical mass of camalexin ($C_{11}H_8N_2S$) is 200.0408. The proton NMR spectrum of the *A. lyrata* compound is nearly identical to that obtained for camalexin isolated from *C. sativa* [16] using deuterated benzene as the solvent. These data provide conclusive evidence that the *A. lyrata* compound is camalexin. It is not surprising that *A. lyrata* and *A. thaliana* produce the same phytoalexin since both plants are closely related to each other [11, 12].

The time course of camalexin accumulation in *A. lyrata* after inoculation with *C. carbonum* (Fig. 2) is similar to that of camalexin accumulation in *A. thaliana* after inoculation with the same fungus [17]. Camalexin accumulation peaks 24–48 h after inoculation. Levels of camalexin were undetectable in noninoculated leaves of *A. lyrata*.

EXPERIMENTAL

Plant material

Plants (*Arabis lyrata*) were grown from seed collected at Saugutuck Dunes State Park (Saugutuck, MI,). Plants were grown in a growth chamber under a 16 h light period at 20°C alternating with an 8 h dark period at 15°C.

Preparation of inoculum

Cochliobolus carbonum (Race 1) was grown on V-8 juice agar [per l: 163 ml V-8 juice, 1 g $CaCO_3$, 14 g agar (Sigma Chemical, St. Louis, MO)]. Detached leaves of *A. lyrata* were inoculated with spores of *C. carbonum* as previously described for inoculation of detached leaves of *A. thaliana* with the same fungus [17]. *Pseudomonas syringae* pv. *maculicola* (*Psm*) strain ES4326 was maintained on LB agar and grown in King's B broth for plant inoculations [17]. When bacterial cells were in logarithmic growth, the cells were harvested by centrifugation (5 min at 3000*g*) and resuspended in 10 mM $MgSO_4$ at an $O.D._{600}$ of 0.015 prior to infiltration into leaves of *A. lyrata*.

Extraction and isolation of camalexin

For large-scale isolation of camalexin, fifty leaves of *A. lyrata* were infiltrated with a bacterial suspension of *Psm* ES4326. Two days after inoculation, the leaves were placed in a 300 ml solution of 80% MeOH in water. The MeOH solution was then

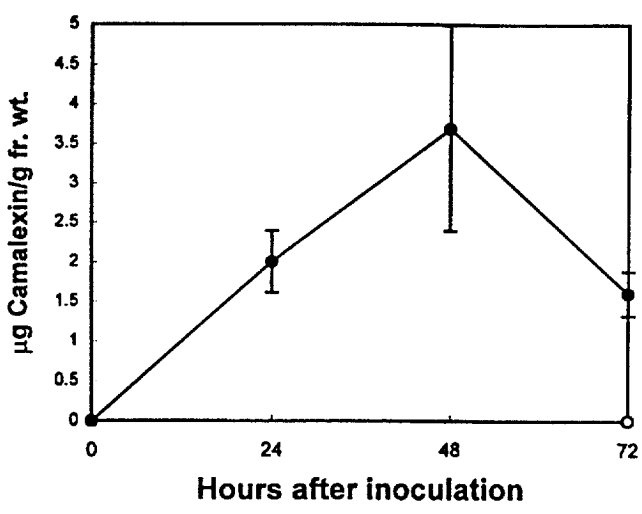

Fig. 2. Accumulation of camalexin in detached leaves of *Arabis lyrata* following inoculation with a *C. carbonum* spore suspension (●) or no treatment (○). The fungal inoculum and determination of the levels of camalexin accumulation are described in the Experimental. All data points represent the mean of three determinations ± S.E. from a single time course.

heated until boiling. After the volume of the solution was reduced by 70% of the original volume, 300 ml of water was added to the methanolic extract and the solution was extracted twice with an equal volume of $CHCl_3$. The pooled $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The remaining residue was dissolved in $CHCl_3$ and applied in separate aliquots to four silica gel G60 TLC plates and developed with $CHCl_3$:MeOH (9:1, v/v). Camalexin was visualized under short-wave UV light and scraped from the silica gel plates into a sintered glass funnel. Camalexin was eluted from the silica gel using EtOAc. Following evaporation of the EtOAc, the remaining residue was redissolved in hexane:*iso*-PrOH (93:7, v/v) just prior to HPLC purification. The HPLC mobile phase [hexane:*iso*-PrOH (93:7, v/v)] was pumped through a 5 μm Alltech Econosphere 150 × 4.6 mm silica column at a flow rate of 1.0 ml/min.*The eluant from the column was monitored at 210 nm using a variable wavelength detector. The camalexin peak in each sample was determined by comparison to a camalexin standard. The pooled camalexin peaks were dried under a stream of nitrogen prior to NMR and mass spectroscopic analysis.

Camalexin assay

The quantitation of camalexin levels in *A. lyrata* leaves following inoculation with spore suspensions of *C. carbonum* was determined as previously described for leaves of *A. thaliana* [17]. In brief, five leaves per sample were extracted in 30 ml of boiling 80% (v/v) MeOH. After the volume of MeOH was reduced to 10 ml, 30 ml of water were added and the extract was extracted twice with an equal volume of $CHCl_3$. The pooled $CHCl_3$ extracts were analyzed by TLC and HPLC as described above.

^{1}H NMR (500 MHz, benzene-d_6) 8.66 (^{1}H, *d*, J = 8.0 Hz, H-4); 7.69 (^{1}H, *d*, J = 3.3, H-4'); 7.31 (^{1}H, *d*, J = 2.8, H-2); 7.26 (^{1}H, *td*, J = 7.28, 1.0, H-5); 7.18 (^{1}H, *td*, J = 8.0,1,1, H-6); 6.94 (^{1}H, *dt*, J = 8.1, 0.9, H-7); 6.61 (^{1}H, *d*, J = 3.3, H-5').

EIMS (70 eV) *m/z* 200.0410 meas, 200.0408 calculated for $C_{11}H_8N_2S$. 200(100), 142(22), 115(10), 86(7), 58(23).

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