

Host-Pathogen Interactions

VIII. ISOLATION OF A PATHOGEN-SYNTHEZIZED FRACTION RICH IN GLUCAN THAT ELICITS A DEFENSE RESPONSE IN THE PATHOGEN'S HOST¹

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ABSTRACT

A polysaccharide from the fungal pathogen *Colletotrichum lindemuthianum* causes browning and phytoalexin production when applied to the cut surfaces of bean (*Phaseolus vulgaris*) cotyledons and hypocotyls. The application of an amount of polysaccharide equivalent to less than 100 ng of glucose will elicit this response in the bean tissues. The polysaccharide has been isolated both from culture filtrates and from the mycelial walls of the fungus. Purification of the polysaccharide involved anion and cation exchange chromatography and gel filtration. The polysaccharide has an apparent molecular weight between 1,000,000 and 5,000,000 daltons, and consists predominantly of 3- and 4-linked glucosyl residues.

Beans synthesize several phytoalexins in response to attack by pathogens (5-7, 28, 33, 38, 42). Four of these phytoalexins have been isolated and characterized as phaseollin, phaseollidin, phaseollinisoflavan, and kievitone (5, 6, 30, 31, 38, 42). The synthesis of these phytoalexins has been demonstrated in beans that have responded to attack by *C. lindemuthianum* (5, 7, 25). The synthesis of the four phytoalexins occurs much sooner in an incompatible reaction than in a compatible reaction. The incompatible reaction usually involves a hypersensitive response in those cells that are initially invaded by the pathogen (5, 28, 37). It has been suggested (5, 7) that the early synthesis of phytoalexins in an incompatible reaction can account for the restricted growth of the pathogen that is characteristic of the hypersensitive response.

Synthesis of phytoalexins in plant tissues can be stimulated by molecules, elicitors (23), that are produced by plant pathogens (12, 23, 24, 34, 43). However, only in one example (12) has the elicitor been purified and partially characterized. This elicitor, a small peptide isolated from the mycelia of *Monilinia fructicola*, is active on tissues of a nonhost of this pathogen, but the effect of the peptide on tissues of the hosts of *M. fructicola* has not been reported.

The synthesis of phytoalexins can also be stimulated by nu-

merous procedures that are apparently unrelated to pathogenesis. These include treatment of the plant tissues with UV light (10, 19) and freezing (32). Phytoalexin synthesis is stimulated, too, by the application to plant tissues of many chemicals such as polyamines (16, 17), antibiotics (4, 35, 36), DNA intercalating agents (18, 20), and salts of heavy metals (11, 34, 35). However, the concentrations of these reagents required to stimulate phytoalexin synthesis (10^{-3} - 10^{-5} M) are higher than those (10^{-8} - 10^{-10} M) required for the pathogen-produced elicitors.

We wish to report the partial purification and characterization of molecules from *C. lindemuthianum* that are capable of eliciting phytoalexin production in bean tissues; these tissues are susceptible to infection by compatible races of this pathogen. The accumulation of phytoalexins in the tissues treated with the elicitor is always accompanied by browning. Browning is also coupled with phytoalexin production during a normal hypersensitive response to an incompatible race of *C. lindemuthianum* (5, 7, 37). These pathogen-produced molecules can elicit host responses that mimic the responses of the host to an attack by an incompatible race of the pathogen.

MATERIALS AND METHODS

Culture of *C. lindemuthianum*. The alpha race of *Colletotrichum lindemuthianum* was maintained at 19 C on 1.5% agar slants containing the medium described by Mathur *et al.* (27). The fungus was propagated by transferring spores onto new slants every two weeks.

The medium for growth of *C. lindemuthianum* in liquid culture contained in 900 ml of water, 15 g of sucrose, 1 g of KH_2PO_4 , 0.25 g of MgSO_4 , 0.027 g of FeCl_3 , and 100 ml of casein hydrolysate extract. The casein hydrolysate extract was prepared as follows: 100 g of casein hydrolysate (Sigma) were dissolved in 1 liter of water and 3 liters of 95% ethanol were added. The mixture was stirred for 30 min. The insoluble material was removed by filtration through Whatman GFC paper. The clear filtrate was reduced to 1000 ml by evaporation under reduced pressure at 40 C to give the concentrated extract used in the medium.

Inoculation of the liquid media involved the sterile transfer of 10^6 spores into 1 liter of the medium, contained in 2800-ml Fernbach flasks. The cultures were grown for 8 days at 23 C on a 2.5-cm radius gyrotary shaker at 100 rpm (14).

Assay of Elicitor Activity. The assay of elicitor activity in preparations from *C. lindemuthianum* depended upon the ability of the elicitor to cause the symptoms of the hypersensitive response in bean tissues. Two of the symptoms of the hypersensitive response were measured. These involved the relative browning of treated tissues and the production of phytoalexins

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in these tissues (5, 7, 37). The activity of the elicitor preparations was determined on both cotyledons and hypocotyls. The Dark Red Kidney variety of *Phaseolus vulgaris* was used for all assays, as the alpha race of *C. lindemuthianum* produces a hypersensitive response with the tissues of this variety (unpublished results of the authors).

Cotyledons and hypocotyls were excised from 7- to 8-day-old plants that had been grown in vermiculite under a 24-hr regime consisting of 14 hr of about 2000 ft-c of illumination at 65 C and 10 hr of darkness at 60 C. The excised cotyledons were surface sterilized by immersion in 1% sodium hypochlorite for 1 min. The sodium hypochlorite was removed by extensive washing in distilled H₂O.

The cotyledons were prepared for the elicitor assay by removing a slice with a razor blade from their convex side so as to provide a flat surface on which the test solution was applied. The hypocotyls were prepared by removing, with the aid of a razor blade, a thin epidermal strip along their length. The cut ends of the hypocotyls were sealed with petroleum jelly. The prepared hypocotyls or cotyledons were then placed in Petri dishes, each of which contained a moistened filter paper.

The extracts to be tested for elicitor activity were supplemented with antibiotics to give a final concentration of 50 μ g/ml of both penicillin and vancomycin. Water containing the same amount of antibiotics was used as a control. One hundred microliters of the solution to be assayed for elicitor activity were placed on each of the cut surfaces of five cotyledons or five hypocotyls. A 5-fold dilution series was used for quantitation purposes. The treated tissues were incubated at 25 C in darkness for 24 hr. After this incubation period, the degree of browning of the surface of the treated tissues was estimated. A scale of +++++ for tissues that were very brown to + for tissues that were only just noticeably brown was used to semiquantitate the activity. An amount of elicitor in 100 μ l that produced a + reaction was defined as one unit of elicitor activity.

The presence of phytoalexins in the test tissues was detected by two techniques. The first technique involved the identification of the phytoalexin, phaseollin, as phaseollin acetate by combined gas chromatography and mass spectrometry (7). The second method, a bioassay, detected phaseollin and other phytoalexins (5, 6). Both assays involved the same procedure for the extraction of phytoalexins from the plant tissues. The treated tissues were homogenized in 95% ethanol using 10 ml of ethanol/g of tissue. The insoluble material in the homogenate was removed by passage through a coarse sintered-glass funnel. The filtrate obtained was evaporated to dryness under reduced pressure at 40 C, and the residue was extracted with 1 ml of ethyl acetate for each 2 g of tissue that was homogenized.

Gas chromatographic analysis of phaseollin in the ethyl acetate extract was initiated by evaporating to dryness, in a small test tube under a stream of nitrogen, the extract from 2 g of tissue. Acetic anhydride (250 μ l) and pyridine (5 μ l) were added to the tube. The tube was sealed and left at room temperature for 24 hr. A 5- μ l portion of the acetylated sample was drawn into a syringe that contained 1 μ l of hexacosane solution (1 mg/ml in chloroform). The hexacosane was used as a retention time standard in the gas chromatographic assay. Gas chromatography was performed on a 183 \times 0.32 cm column containing 3% OV 1 on Chromasorb W (HP). The 6- μ l sample was injected into the column at 200 C. The column was maintained at 200 C for 5 min and then increased from 200 C to 250 C at a rate of 2 C/min. Helium, flowing at approximately 60 ml/min, was used as the carrier gas.

The bioassay for phytoalexin detection (5, 6) also used the dried residue from the ethyl acetate extract of 2 g of tissue. The

residue was dissolved in 50 μ l of ethyl acetate and spots of 2 and 5 μ l were applied to a thin layer silica gel chromatography plate. The silica plate was developed in a mixture of chloroform and methanol (100:4) and then air-dried. For the bioassay, a suspension of *Cladosporium cucumerinum* spores was prepared by adding water to a mat of the fungus that had been grown for 5 to 8 days at room temperature on 10-cm Petri dishes containing potato dextrose agar (Difco). The surface of the fungal mat was rubbed gently to release the spores. This aqueous suspension of spores was mixed with an equal volume of a solution of potato dextrose agar at 50 C. The mixture of spores in the nutrient agar solution was sprayed onto the silica plates to give an even coating. The coated plates were transferred to a humid incubation box for 2 to 4 days at room temperature. After this time, any regions that lacked fungal growth appeared as white areas on the dark background of the fungal mat. The white areas corresponded to the positions of the phytoalexins which inhibited the growth of *C. cucumerinum*.

Assay of Monosaccharide Composition of Elicitor Preparations by Using Gas Chromatographic Analysis of Corresponding Alditol Acetates. Aliquots of elicitor preparations containing 100- μ g equivalents of glucose were hydrolyzed and the alditol acetates prepared and analyzed by the method of Jones and Albersheim (21).

Methylation Analysis of Glycosyl Linkage Composition in Elicitor Preparations. Aliquots of elicitor preparations containing from 500- to 1000- μ g equivalents of glucose were methylated using the method described by Talmadge *et al.* (41). The methylated polymer was hydrolyzed and the partially methylated monosaccharides were reduced, acetylated, and analyzed by the procedures described (41).

RESULTS

Isolation of Elicitor from Culture Filtrates of *C. lindemuthianum*. Ten 8-day-old, 1-liter cultures of *C. lindemuthianum* were filtered through coarse sintered-glass funnels. The extracellular media obtained by this procedure was concentrated to 1 liter by passage through a Bio-Rad mini plant 80 device which restricts the passage of molecules of size greater than 30,000 daltons. Three liters of 95% ethanol were added to 1 liter of concentrated culture filtrate. The resulting suspension remained at 4 C for 3 days in order to permit the precipitate to settle. The clear supernatant liquid was siphoned off and discarded. The remaining liquid was removed by centrifugation. The pellet was resuspended in 50 ml of H₂O. The resulting suspension was dialyzed against 10 liters of deionized H₂O for 20 hr. Any insoluble material remaining in the extract after this dialysis was removed by centrifugation. The supernatant liquid was concentrated to 10 ml by evaporation under reduced pressure at 40 C. This concentrated solution was assayed for hexose by the anthrone method (13) and for protein by the procedure of Lowry *et al.* (26). The preparation from 10 liters of culture filtrate contained, in 10 ml, 19-mg equivalents of glucose and 20 mg of protein. This concentrated solution, when assayed for elicitor activity, produced marked browning of the cut bean cotyledons and hypocotyls. A 5000-fold dilution of 1 ml of the concentrated extract produced an activity that was just detectable on the browning scale (+). Thus, 1 liter of 8-day-old *C. lindemuthianum* culture filtrate contains about 50,000 units of elicitor activity.

The two assay procedures described demonstrate that phytoalexins were present in the brown tissues. Phaseollin was identified as phaseollin acetate in the gas liquid chromatographic assay by comparison with an authentic sample of phaseollin acetate. This was prepared from a sample of phaseollin kindly

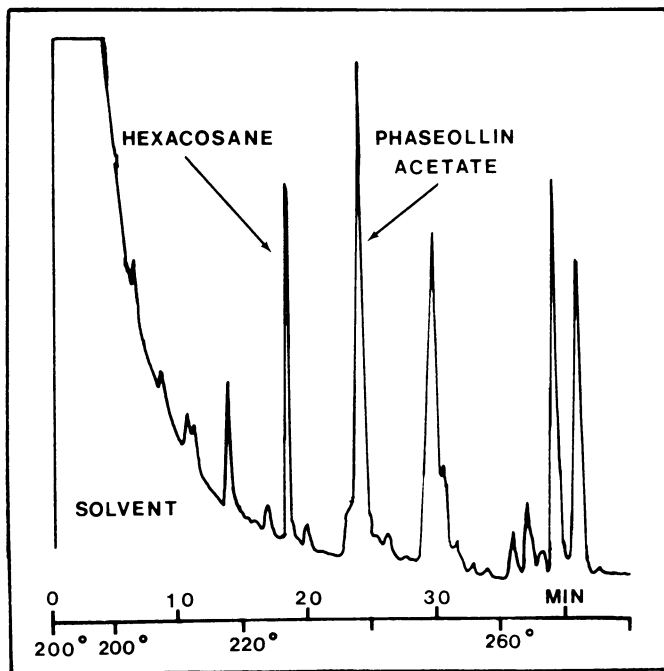


FIG. 1. Gas chromatogram of the acetylated derivatives from the ethyl acetate extract of elicitor-treated bean cotyledons.

provided by H. van Etten (Cornell University). The identity of phaseollin acetate was confirmed by mass spectral analysis of the gas chromatographic output (R. H. Shapiro, personal communication). In our gas chromatographic assay, the peak of phaseollin acetate eluted from the column at 1.31 times the retention of the standard, hexacosane (Fig. 1).

Phaseollin, phaseollidin, phaseollinisoflavan, and kievitone were also tentatively identified in ethyl acetate extracts of elicitor-treated tissues by co-chromatography with authentic samples of these phytoalexins on thin layer plates. The phytoalexins were detected on the plates by the assay described. Authentic samples of these additional phytoalexins were also the gift of H. van Etten.

No browning was observed when bean tissues were treated with water, containing antibiotics, as a control. The two assay procedures described failed to detect the presence of any phytoalexins in these control tissues. The application of a preparation of uninoculated culture medium, concentrated by the same procedures as that described for the preparation of elicitor, also produced no browning or phytoalexin production in the bean tissues.

Isolation of Elicitor from *C. lindemuthianum* Mycelial Walls. Mycelia from 8-day-old liquid cultures were homogenized in a Waring blender for 60 sec using 5 ml of water per g wet weight of mycelia. The homogenate was filtered through a coarse sintered-glass funnel, and the residue obtained on the filter was saved. The residue was homogenized three times more in water, once in a mixture of chloroform and methanol (1:1), and finally in acetone. This preparation, when air dried, represents the fraction referred to as the mycelial walls.

Elicitor was extracted from the mycelial walls by suspending 1 g of walls in 100 ml of H₂O and autoclaving for 20 min at 121 C. The autoclaved suspension was filtered through a coarse sintered-glass funnel. The filtrate was then clarified by centrifugation and concentrated to 10 ml under reduced pressure. This concentrated solution contained in 10 ml, 12-mg equivalents of glucose and 15 mg of protein. The application of this extract to cotyledons and hypocotyls produced marked

browning and resulted in the synthesis in the tissues of the four phytoalexins identified above. A 5000-fold dilution of the 1 ml of the concentrated extract produced an activity that was just detectable (+) in the cotyledon assay.

Purification of Elicitor. The crude elicitor from either the culture filtrate or the mycelial walls was purified by chromatography on a sizing column (G-175 glass beads [Sigma]) and by passage through a cation (AG 50 W [Bio Rad]) and an anion (AG 1X1 [Bio Rad]) exchange column.

Purification of the elicitor by gel filtration was accomplished by applying an aliquot (0.4–1.0 ml) of crude elicitor on a G-175 glass bead column (55 × 1.4 cm). Degassed H₂O was passed through the column and 2-ml fractions were collected. Each fraction was assayed for elicitor activity using the cotyledon assay, for hexose, and for protein. The void and inclusion volumes of the column were determined from the elution pattern of blue dextran (Sigma) and glucose, respectively. The majority of the hexose and protein applied in the elicitor preparation eluted in the void fractions. All of the elicitor activity was detected in the void fractions. This indicated that the elicitor behaves like a dextran with a molecular size, under these conditions, of at least 60,000 daltons. Fractions with elicitor activity were combined. Further aliquots of the crude concentrated preparations were passed through the sizing column until all of the extract had been chromatographed. The pooled fractions containing the elicitor were concentrated to 10 ml under reduced pressure at 40 C. This concentrated solution was adjusted to 20 mM HCl by the addition of 200 mM HCl.

The elicitor present in the acidified solution was further purified by passage through a column of AG 50 W cation exchange resin (15 × 1.6 cm) that had been equilibrated in 20 mM HCl. The column was washed with 50 ml of 20 mM HCl and the wash combined with the initial eluate. The combined fractions were dialyzed against H₂O and assayed for elicitor activity, hexose, and protein. Elicitor activity and most of the hexose passed through the column, whereas some of the protein was retained (Table I).

The elicitor was markedly further purified by passage through an anion exchange resin. The AG 50 W eluate was concentrated to 10 ml under reduced pressure at 40 C. The solution was then adjusted to 20 mM glycine-NaOH buffer, pH 10, and passed through a column containing AG 1X1 resin (15 × 1.6 cm) that had been equilibrated in the 20 mM glycine-

Table I. Summary of Purification of Extracellular Elicitor Synthesized by *Colletotrichum lindemuthianum*.

The data represent the elicitor activity obtained from 10 liters of culture medium. Details of the purification procedure are described in "Results." One unit of elicitor activity is that amount which when applied as a 100- μ l sample to the cut surface of a cotyledon will just produce noticeable browning after 24 hours incubation at 25 C.

Elicitor Preparation	Total Glucose Equivalents	Total Protein	Elicitor		
	μ g	μ g	units $\times 10^{-3}$	units/ μ g protein	units/ μ g glucose equivalent
Crude	18,875	20,460	550	27.0	29.3
G175 eluate	15,900	17,400	530	30.4	33.3
AG 50 W, 20 mM eluate	12,600	8,000	500	62.5	39.6
AG 1X1, 20 mM eluate	1,300	20	400	20,000	310.0
AG 1X1, 200 mM eluate	6,000	1,000	0	0	0.0

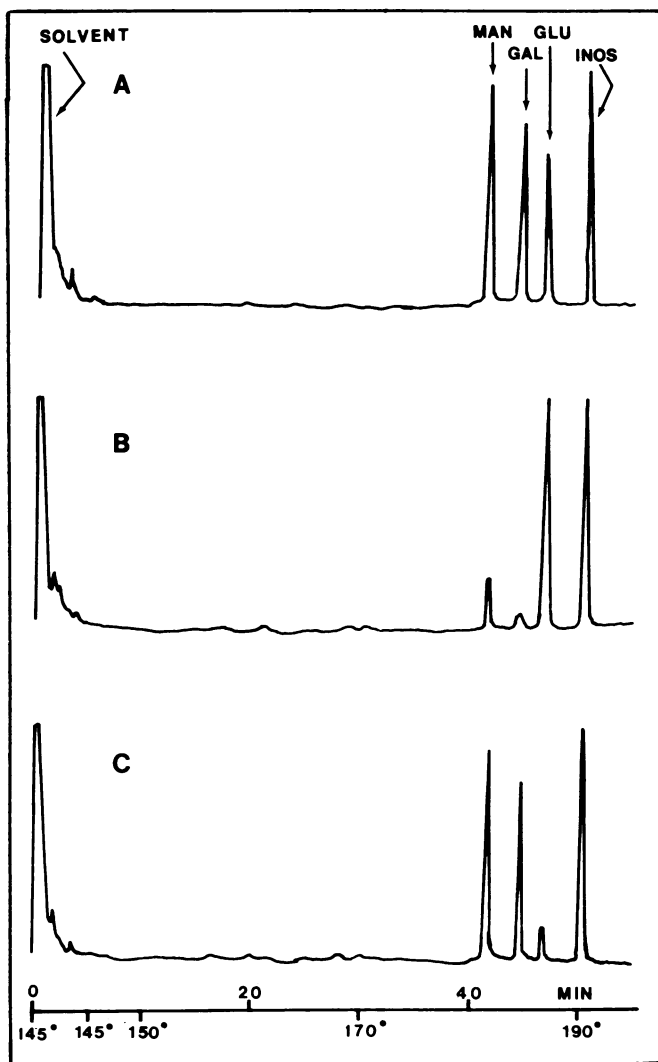


FIG. 2. Gas chromatograms of the alditol acetates derived from various preparations of *C. lindemuthianum* culture filtrate. The purification procedures used are described in "Results." Chromatogram A is from a crude preparation containing elicitor activity. Chromatogram B is from the 20 mM glycine-NaOH, pH 10, eluate of the AG 1X1 column. Chromatogram C is from the 200 mM glycine-NaOH, pH 10, eluate of the same AG 1X1 column. The alditol acetate derivatives were prepared and analyzed by methods previously described (21). Sugars detected: MAN, mannose; GAL, galactose; GLU, glucose; and INOS, inositol which was added as an internal standard.

NaOH buffer. The eluate was combined with a 100-ml wash of the column with the same buffer. The column was then washed with 250 ml of 200 mM glycine-NaOH buffer, pH 10. The eluates from the AG 1X1 column were extensively dialyzed against H₂O and then assayed for elicitor activity, hexose, and protein. Almost all of the elicitor activity passed through the column, whereas most of the hexose and protein were bound by the resin (Table I). The 200 mM buffer eluate lacked elicitor activity but did contain some of the hexose and protein. The purified elicitor preparation obtained from the culture filtrate by this sequence of sizing and ion exchange columns contained carbohydrate and only traces of material giving a positive protein assay (Table I).

The elicitor released from the mycelial walls behaved in a manner identical to that of the culture-filtrate elicitor when

subjected to the same purification procedure. The purified preparation obtained from 1 g of mycelial walls contained, in 5 ml, 3 mg equivalents of glucose and 0.05 mg of protein. This preparation gave an activity that was just detectable on the cotyledon assay (+) when 1 ml was diluted 10,000-fold.

Determination of Glycosyl Constituents and Their Linkages in *C. lindemuthianum* Elicitor Preparations. Acetylation analysis of the monosaccharides present in the elicitor preparation was performed at several stages of the purification procedures (Fig. 2). Impure preparations of elicitor contained more galactose and mannose than glucose (Fig. 2A). However, the purest elicitor preparations contained predominantly glucose (Fig. 2B). Polymers containing mannose and galactose were absorbed onto the AG 1X1 anion exchange resin and could be eluted by buffer of higher ionic strength (Fig. 2C). These polymers possessed no elicitor activity.

Methylation analysis of our most purified preparations of elicitor from *C. lindemuthianum* culture filtrates showed that the glycosyl residues were predominantly 3- and 4-linked (Fig. 3). Previously, Albersheim and Valent (2) have shown that similarly linked glycosyl residues are the predominant glycosyl constituents of the mycelial walls of *C. lindemuthianum* (Fig. 3).

Properties of *C. lindemuthianum* Elicitor. The highly purified preparations of elicitor, isolated from either mycelial walls or culture filtrate, were exceedingly active. The application to the surface of cut cotyledons of an amount of purified elicitor equivalent to less than 10⁻⁷ g of glucose caused noticeable browning of the tissue. All four phytoalexins were detected in organic solvent extracts from cotyledons treated in this manner. The specificity of this response is also demonstrated by the failure of solutions of glucose, starch, or laminarin (29) to induce browning or phytoalexin production when these substances were applied to cotyledons in amounts equivalent to 10⁻³ g of glucose.

The highly purified elicitor is stable at high temperatures. Autoclaving an aqueous solution of the elicitor at 121 C for 2 hr does not alter its activity. The elicitor was also stable to prolonged exposure to pH 2 and pH 10, a property which was utilized in the purification procedure.

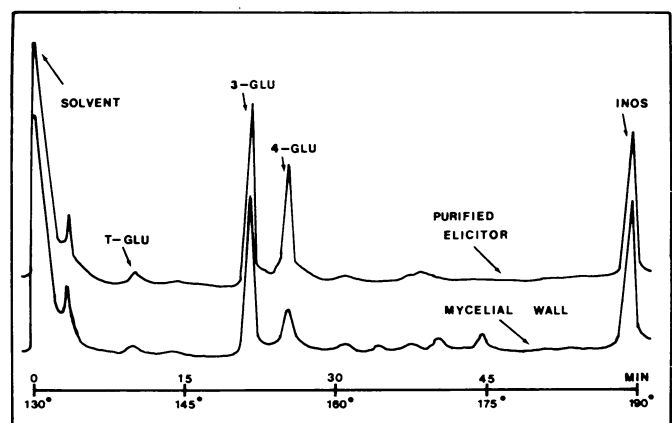


FIG. 3. Gas chromatograms of the partially methylated alditol acetates derived from *C. lindemuthianum* mycelial walls and from a highly purified elicitor preparation. The isolation of the mycelial walls and the purification procedure for the elicitor is described in "Results." The glycosyl derivatives were prepared and analyzed by methods described (41). Glycosyl linkages in the polysaccharides: 3-GLU, 3-linked glycosyl residues; 4-GLU, 4-linked glycosyl residues; and T-GLU. Inositol (INOS) was added as an internal standard.

Gel filtration of the highly purified elicitor on Bio-Gel A-5m in 0.3 M NaCl indicated that about 60% of the elicitor was sufficiently large to void the column. The remaining elicitor molecules were slightly included. Dextran of average mol wt of 2,000,000 daltons (Sigma) gave an elution pattern almost identical to that of the elicitor. Under the conditions employed for chromatography, the elicitor molecules appear to be heterogeneous in size with an apparent minimum mol wt between 1,000,000 and 5,000,000 daltons.

The highly purified preparations of elicitor were not degraded by prolonged incubation with a purified endo- β -1,3-glucanase that was isolated from bean tissues following the procedure of Abeles *et al.* (1). However, degradation of the elicitor to fragments of lower mol wt which do not have elicitor activity was observed upon incubation of elicitor with a partially purified enzyme preparation from *Trichoderma viride* (9) which possesses both β -1,4- and β -1,3-endoglucanase activity (A. J. Anderson-Prouty, unpublished results).

DISCUSSION

The possibility that pathogens produce molecules which are able to stimulate phytoalexin production in plant tissues (23) is confirmed by our studies with *C. lindemuthianum*. *C. lindemuthianum* produces neutral polymeric molecules which are stable to heat, acid, and alkali, and which can cause tissues of its host, the bean, *Phaseolus vulgaris*, to turn brown and to synthesize phytoalexins. The elicitor-treated tissues synthesize the same phytoalexins as those found upon infection of beans by *C. lindemuthianum* and other plant pathogens.

Our observations that culture filtrates of *C. lindemuthianum* contain heat stable molecules that can cause browning in bean hypocotyls confirm the observations of Skipp and Deverall (37) and of Mercer *et al.* (28). Skipp and Deverall (37) showed that application of the culture filtrate to bean tissues caused the treated tissues to become resistant to otherwise virulent races of *C. lindemuthianum*. This resistance was attributable to failure of the otherwise virulent spores to germinate on the pretreated tissues. Ultrastructural studies on the culture filtrate-treated tissues by Mercer *et al.* (28) showed changes similar to those observed during a hypersensitive response initiated by the incompatible interaction of a race of *C. lindemuthianum* with a bean variety resistant to that race. Because phytoalexin accumulation is another phenomenon of the hypersensitive response (5, 7), our demonstration that phytoalexins accumulate in elicitor-treated tissues confirms that these molecules can initiate the symptoms of an incompatible interaction. Presumably, Skipp and Deverall (37) were observing, in their culture filtrate-treated tissues, the effect of molecules that we term elicitors.

Gel filtration of the *C. lindemuthianum* elicitor showed that the active molecules are heterogeneous in size with an apparent minimum mol wt of 1,000,000 daltons. The most highly purified preparations consist almost entirely of carbohydrate in which glucose is the predominant monosaccharide. This shows that the elicitor is distinct from the mycelial wall and culture filtrate polymers that contain mainly mannose and galactose. Methylation analysis demonstrated that the glucosyl residues of the purified elicitor are predominantly 3- and 4-linked. Preliminary evidence using impure mixtures of polysaccharide-degrading enzymes suggests that the glucosyl residues are predominantly β -linked. Glucans containing 3- and 4-linked residues are also present in the mycelial walls of *C. lindemuthianum* (2). However, the ratio of 3- and 4-linked glucosyl residues in the elicitor is somewhat different from that observed in the total mycelial wall glucans (Fig. 3).

It is impossible to determine from the available data whether

the quantitatively minor sugars (2% or less of the total sample) in the purified elicitor preparations are an integral part of the elicitor, comprise the bulk of the elicitor, or are contaminants that have not been separated by the purification procedures employed. However, the possible existence in the elicitor of mannosyl and galactosyl residues has intriguing implications. Ballou (8) has shown in his studies of yeast cell wall polysaccharide-protein complexes that quantitatively minor glycosyl derivatives determine the antigenic specificity of these molecules and, indeed, of the yeast cells themselves. It seems possible that the biological activity of elicitors may be determined by the quantitatively minor constituents of these polysaccharides.

The finding that a host plant can recognize a pathogen-produced glucan and, as a result, initiate phytoalexin production, is also observed in another unrelated system. Ayers *et al.* (3) have demonstrated that glucans from *Phytophthora megasperma* var. *sojae* will initiate phytoalexin synthesis in tissues of the host of this fungus, the soybean, *Glycine max*. These glucans from *P. megasperma* var. *sojae* resemble glucans that are found in the mycelial walls of that fungus. Also, like the *C. lindemuthianum* elicitor, the *P. megasperma* var. *sojae* elicitor is extremely active at inducing phytoalexin synthesis. Phytoalexin synthesis in the soybean is activated when *P. megasperma* elicitor equivalent to less than 0.1 μ g of glucose is applied to the cut surface of a soybean cotyledon. Thus, in two systems, wall-like polysaccharides from pathogenic fungi are able to stimulate the tissues of their host plants to initiate the synthesis of phytoalexins. As phytoalexins are inhibitory to the growth of fungi (25), this response in the plant can be considered as a defense mechanism against the attacking pathogen.

The observation that high mol wt polysaccharides of pathogen origin are active as inducers of phytoalexin synthesis in plants is a second example of the importance of these types of molecules in plant pathogenesis. Pathogen-synthesized high mol wt molecules which are largely polysaccharide in nature have been demonstrated to be toxic to plants (15, 40). The toxic polysaccharides can cause many of the symptoms of the pathogen's disease in the absence of the pathogen (40).

Some of the high mol wt polysaccharide toxins have a limited range of plants that they can affect (15, 39, 40). It is possible that elicitors are similarly specific. However, at present there is no indication that varietal specificity in the *C. lindemuthianum* system can be attributed to elicitor activity. Lack of specificity may be inherent in the bioassay employed for the detection of elicitor activity (22). It is also possible that the chemical nature of the elicitor has been altered to cause it to lose varietal specificity. This alteration may occur enzymically during fungal culture, or chemically during the purification procedure. Regardless of whether the elicitor is race specific, the low concentrations at which the elicitor is active and the failure of other glucans to initiate phytoalexin synthesis suggest that the elicitor does have biological significance.

LITERATURE CITED

1. ABELES, F. B., R. P. BOSSHART, L. E. FORRENCE, AND W. H. HABIG. 1970. Preparation and purification of glucanase and chitinase from bean leaves. *Plant Physiol.* 47: 129-134.
2. ALBERSHEIM, P. AND B. S. VALENT. 1974. Plant pathogens secrete proteins which inhibit enzymes of the host capable of attacking the pathogen. *Plant Physiol.* 53: 684-687.
3. AYERS, A., J. EBEL, AND P. ALBERSHEIM. 1974. A highly potent elicitor of hydroxyphaseollin synthesis in soybean tissues has been purified from the culture filtrates of *Phytophthora megasperma* var. *sojae*. *Proc. Am. Phytopathol. Soc.* 1: 23.
4. BAILEY, J. A. 1969. Effects of antimetabolites on production of the phytoalexin pisatin. *Phytochemistry* 8: 1393-1395.
5. BAILEY, J. A. 1974. The relationship between symptom expression and

- phytoalexin concentration in hypocotyls of *Phaseolus vulgaris* infected with *Colletotrichum lindemuthianum*. *Physiol. Plant Path.* 4: 477-488.
6. BAILEY, J. A. AND R. S. BURDEN. 1973. Biochemical changes and phytoalexin accumulation in *Phaseolus vulgaris* following cellular browning caused by tobacco necrosis virus. *Physiol. Plant Path.* 3: 171-177.
 7. BAILEY, J. A. AND B. J. DEVERALL. 1971. Formation and activity of phaseollin in the interaction between bean hypocotyls (*Phaseolus vulgaris*) and physiological races of *Colletotrichum lindemuthianum*. *Physiol. Plant Path.* 1: 435-449.
 8. BALLOU, C. E. 1974. Some aspects of the structure, immunochemistry, and genetic control of yeast mannans. *Adv. Enzymol.* 40: 239-270.
 9. BAUER, W. D., K. W. TALMADGE, K. KEEGSTRA, AND P. ALBERSHEIM. 1973. The structure of plant cell walls. II. The hemicellulose of the walls of suspension-cultured sycamore cells. *Plant Physiol.* 51: 174-187.
 10. BRIDGE, M. A. AND W. L. KLARMAN. 1973. Soybean phytoalexin, hydroxy-phaseollin, induced by ultraviolet irradiation. *Phytopathology* 63: 606-609.
 11. CRUICKSHANK, I. A. M. AND D. R. PERRIN. 1963. Studies on phytoalexins. VI. Pisatin: the effect of some factors on its formation in *Pisum sativum* L., and the significance of pisatin in disease resistance. *Aust. J. Biol. Sci.* 16: 111-128.
 12. CRUICKSHANK, I. A. M. AND D. R. PERRIN. 1968. The isolation and partial characterization of monilicolin A, a polypeptide with phaseollin-inducing activity from *Monilinia fructicola*. *Life Sci.* 7: 449-458.
 13. DISCHE, Z. 1962. Color reaction of carbohydrates. In: R. L. Whistler and M. L. Wolfrom, eds., *Methods in Carbohydrate Chemistry*, Vol. 1. Academic Press, New York, pp. 477-512.
 14. ENGLISH, P. D. AND P. ALBERSHEIM. 1969. Host-pathogen interactions. I. A correlation between α -galactosidase production and virulence. *Plant Physiol.* 44: 217-224.
 15. GOODMAN, R. N., J. S. HUANG, AND P.-Y. HUANG. 1974. Host-specific phyto-toxic polysaccharide from apple tissue infected by *Erwinia amylovora*. *Science* 183: 1081-1083.
 16. HADWIGER, L. A., A. JAFRI, S. VON BROEMSEN, AND R. EDDY, JR. 1974. Mode of pisatin induction. Increased template activity and dye-binding capacity of chromatin isolated from polypeptide-treated pea pods. *Plant Physiol.* 53: 52-63.
 17. HADWIGER, L. A. AND M. E. SCHWOCHAU. 1970. Induction of phenylalanine ammonia lyase and pisatin in pea pods by poly-lysine, spermidine or histone fractions. *Biochem. Biophys. Res. Commun.* 38: 683-691.
 18. HADWIGER, L. A. AND M. E. SCHWOCHAU. 1971. Specificity of deoxyribonucleic acid intercalating compounds in the control of phenylalanine ammonia lyase and pisatin levels. *Plant Physiol.* 47: 346-351.
 19. HADWIGER, L. A. AND M. E. SCHWOCHAU. 1971. Ultraviolet light-induced formation of pisatin and phenylalanine ammonia lyase. *Plant Physiol.* 47: 588-590.
 20. HESS, S. L. AND L. A. HADWIGER. 1971. The induction of phenylalanine ammonia lyase and phaseollin by 9-aminoacridine and other deoxyribonucleic acid intercalating compounds. *Plant Physiol.* 48: 197-202.
 21. JONES, T. M. AND P. ALBERSHEIM. 1972. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharides. *Plant Physiol.* 49: 926-936.
 22. KEEN, N. T. AND R. HORSCH. 1972. Hydroxyphaseollin production by various soybean tissues, a warning against use of unnatural host-parasite systems. *Phytopathology* 62: 439-442.
 23. KEEN, N. T., J. E. PARTRIDGE, AND A. I. ZAKI. 1972. Pathogen-produced elicitor of a chemical defense mechanism in soybeans monogenically resistant to *Phytophthora megasperma* var. *sojae*. *Phytopathology* 62: 768.
 24. KLARMAN, W. L. AND J. W. GERDEMANN. 1963. Resistance of soybeans to three *Phytophthora* species due to the production of a phytoalexin. *Phytopathology* 53: 1317-1320.
 25. KUĆ, J. 1972. Phytoalexins. *Annu. Rev. Phytopathol.* 10: 207-232.
 26. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
 27. MATHUR, R. S., H. L. BARNETT, AND V. G. LILLY. 1949. Sporulation of *Colletotrichum lindemuthianum* in culture. *Phytopathology* 40: 104-114.
 28. MERCER, P. C., R. K. S. WOOD, AND A. D. GREENWOOD. 1974. Resistance to anthracnose of French bean. *Physiol. Plant Path.* 4: 291-306.
 29. PERCIVAL, E. 1970. Algal polysaccharides. In: W. Pigman and D. Horton, eds., *The Carbohydrates*. Academic Press, New York, pp. 537-568.
 30. PERRIN, D. R. 1964. The structure of phaseollin. *Tetrahedron Lett.* 1: 29-35.
 31. PERRIN, D. R. AND C. P. WHITTLE. 1972. The structure of phaseollidin. *Tetrahedron Lett.* 17: 1673-1676.
 32. RAJE, J. E. 1974. Accumulation of phaseollin in hypocotyls of etiolated *Phaseolus vulgaris* in response to mechanical injury. *Proc. Am. Phytopathol. Soc.* 1: 64.
 33. RAJE, J. E., J. KUĆ, C. M. CHUANG, AND E. B. WILLIAMS. 1969. Correlation of phenolic metabolism with histological changes. *Neth. J. Plant Pathol.* 75: 58-71.
 34. RATHMELL, W. G. AND D. S. BENDALL. 1971. Phenolic compounds in relation to phytoalexin biosynthesis in hypocotyls of *Phaseolus vulgaris*. *Physiol. Plant Pathol.* 1: 351-362.
 35. SCHWOCHAU, M. E. AND L. A. HADWIGER. 1968. Stimulation of pisatin production in *Pisum sativum* by actinomycin D and other compounds. *Arch. Biochem. Biophys.* 126: 731-733.
 36. SCHWOCHAU, M. E. AND L. A. HADWIGER. 1969. Regulation of gene expression by actinomycin D and other compounds which change the conformation of DNA. *Arch. Biochem. Biophys.* 134: 34-41.
 37. SKIPP, R. A. AND B. J. DEVERALL. 1973. Studies on cross-protection in the anthracnose disease of bean. *Physiol. Plant Pathol.* 3: 299-313.
 38. SMITH, D. A., H. D. VAN ETTEN, J. W. SERUM, T. M. JONES, D. F. BATEMAN, T. H. WILLIAMS, AND D. L. COFFEN. 1973. Confirmation of the structure of kievitone, and antifungal isoflavanone isolated from *Rhizoctonia*-infected bean tissues. *Physiol. Plant Pathol.* 3: 293-297.
 39. STRALEY, C. S., M. L. STRALEY, AND G. A. STROBEL. 1974. Rapid screening for bacterial wilt resistance in alfalfa with a phytotoxic glycopeptide from *Corynebacterium insidiosum*. *Phytopathology* 64: 194-196.
 40. STROBEL, G. A. 1974. Phytotoxins produced by plant parasites. *Annu. Rev. Plant Physiol.* 25: 541-566.
 41. TALMADGE, K. W., K. KEEGSTRA, W. D. BAUER, AND P. ALBERSHEIM. 1973. The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. *Plant Physiol.* 51: 158-173.
 42. VAN ETTEN, H. D. AND D. F. BATEMAN. 1970. Isolation of phaseollin from *Rhizoctonia*-infected bean tissue. *Phytopathology* 60: 385-386.
 43. VARNIS, J. L., W. W. CURRIER, AND J. KUĆ. 1971. Specificity of rishitin and phytuberin accumulation by potato. *Phytopathology* 61: 968-971.