MONTANA STATE UNIVERSITY

Chemical and biological properties of a phytotoxic glycopeptide from Corynebacterium insidiosum
by Stephen Michael Ries

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements of the degree of
DOCTOR OF PHILOSOPHY in Microbiology
Montana State University
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Abstract:
Phytotoxins produced by Corynebacterium spp. have been reported by several investigators. However, in the case of a toxin produced by C. insidiosum, the complete chemical, physical, and biological characterization has not been established. The present study demonstrates that the toxin of C. insidiosum is a glycopeptide with an empirical formula of C108H2260132N based on one atom of nitrogen. Chemical analysis shows the toxin is 83.1% polysaccharide composed of mannose (1), glucose (5), galactose (5), and L-fucose (10), with trace amounts of rhamnose and an unidentified reducing sugar. An unknown organic acid comprises 8.8% of the toxin and a single peptide composed of lysine2, arginine1, aspartic acid1, threonine1, serine1, glutamic acid1, glycine2, alanine2, valine2, leucine2, and isoleucine1 accounts for 2.57% of the toxin molecule. The toxin has a blue chromophore due to copper chelation at a concentration of 75 moles copper/mole toxin. Sepharose 2B column chromatography, analytical ultracentrifugation, and light scattering data indicates that the glycopeptide has a molecular weight of 5 X 10 6 daltons. Electron micrographs of the glycopeptide shows it to be an amorphous globule 80.0 - 90.0 mμ in diameter. The toxin has a specific optical rotation of -166°, an intrinsic viscosity of 0.2307 dl/g,. and decomposes at 260°. The toxin causes wilt in both leaves and stems of test plants and exhibits no specificity between alfalfa or tomato seedlings. Biological activity of the compound is dependent on time and on concentration. The toxin is stable to wide fluctuations in pH and still retains biological activity after heating to 121° for 2 hours. Partial acid hydrolysis results in a drastic reduction of biological activity. The mechanism of action of the toxic glycopeptide is unknown but it may cause plugging of xylem vessels or membrane damage.

CHEMICAL AND BIOLOGICAL PROPERTIES OF A PHYTOTOXIC GLYCOPEPTIDE FROM *CORYNEBACTERIUM INSIDIOSUM*

by

STEPHEN MICHAEL RIES

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements of the degree

of

DOCTOR OF PHILOSOPHY

in

Microbiology

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March, 1971

ACKNOWLEDGEMENTS

I wish to take this opportunity to express my sincere appreciation and gratitude to Dr. Gary A. Strobel for his encouragement, enthusiasm, and guidance in my research, as well as in my graduate career.

I wish also to thank Dr. W. Hess for his electron microscopy work, Dr. J. Robbins for use of his analytical ultracentrifuge, Dr. E. Anacker for assistance with the light scattering data and calculations, and Dr. K. Hapner for the amino acid analyses.

I would also like to thank Dr. G. A. Strobel, Dr. J. W. Jutila, Dr. N. M. Nelson, Dr. S. Rogers, and Dr. J. Robbins for their help in the preparation of this manuscript.

I am grateful to Darlene Harpster who typed this manuscript.

Appreciation is also given to the Public Health Service trainee grant 5 TOI AI00131 for financial support throughout this study.

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ABSTRACT

Phytotoxins produced by Corynebacterium spp. have been reported by several investigators. However, in the case of a toxin produced by C. insidiosum, the complete chemical, physical, and biological characterization has not been established. The present study demonstrates that the toxin of C. insidiosum is a glycopeptide with an empirical formula of $C_{108}H_{226}O_{132}N$ based on one atom of nitrogen. Chemical analysis shows the toxin is 83.1% polysaccharide composed of mannose (1), glucose (5), galactose (5), and L-fucose (10), with trace amounts of rhamnose and an unidentified reducing sugar. An unknown organic acid comprises 8.8% of the toxin and a single peptide composed of lysine$_2$, arginine$_1$, aspartic acid$_1$, threonine$_1$, serine$_1$, glutamic acid$_1$, glycine$_2$, alanine$_2$, valine$_2$, leucine$_2$, and isoleucine$_1$ accounts for 2.57% of the toxin molecule. The toxin has a blue chromophore due to copper chelation at a concentration of 75 moles copper/mole toxin. Sepharose 2B column chromatography, analytical ultracentrifugation, and light scattering data indicates that the glycopeptide has a molecular weight of 5×10^6 daltons. Electron micrographs of the glycopeptide shows it to be an amorphous globule 80.0 - 90.0 mμ in diameter. The toxin has a specific optical rotation of -166°, an intrinsic viscosity of 0.2307 dl/g, and decomposes at 260°. The toxin causes wilt in both leaves and stems of test plants and exhibits no specificity between alfalfa or tomato seedlings. Biological activity of the compound is dependent on time and on concentration. The toxin is stable to wide fluctuations in pH and still retains biological activity after heating to 121° for 2 hours. Partial acid hydrolysis results in a drastic reduction of biological activity. The mechanism of action of the toxic glycopeptide is unknown but it may cause plugging of xylem vessels or membrane damage.

INTRODUCTION

Bacterial wilt of alfalfa (Medicago sativa L.), caused by Corynebacterium insidiosum (McCull.) Jensen, is distributed widely in North America and is probably the most important malady of the crop in the United States. Alfalfa plants are killed so rapidly that fields are unprofitable after 3 or 4 years. The typical symptoms of this disease are reduction in vigor of the plants, yellowing and bleaching of the leaves, and finally wilting and death of the plants. The taproot shows a pale-brown discoloration of the outer woody tissue.

Phytotoxic polysaccharides from some plant pathogenic bacteria exert their effect on plants by causing wilting. Hodgson, Peterson, and Riker (22, 23) and Feder and Ark (12) reported that certain polysaccharides produced by plant pathogenic bacteria, Agrobacterium tumefaciens (Erw. Smith and Town) Conn, Xanthomonas phaseoli (Erw. Smith) Dowson, and Erwinia carotovora (Jones) Holland, were phytotoxic and possessed wilt-inducing properties. These toxins were non-specific and of high molecular weight.

Further work on toxins produced by Pseudomonas solanacearum (Erw. Smith) Erw. Smith (24), the causal agent of southern bacterial wilt of several plants, indicated that the culture filtrate contained pectolytic and cellulolytic enzymes. The heat-treated culture filtrate contained material which also caused wilting in tomato and tobacco cuttings. This non-enzymatic, heat-stable material was thought to cause plugging in the vessels. Stewart, in 1894 (10), stated that wilting symptoms result from plugging of conductive elements by the pathogen or its products. The mechanical plugging theory has been supported by many investigators working on various bacterial wilt diseases, including wilt of cucumber caused by _Erwinia tracheiphila_, (Erw. Smith) Holland, wilt of sweet corn caused by _Xanthomonas stewarti_ (Erw. Smith) Dowson, wilt of carnation caused by _Pseudomonas caryophilli_ (Burkholder) Starr and Burkholder (7) and several others. However, in the case of _Pseudomonas solanacearum_, Hutchinson in 1931 (7) reported that wilting was due to alteration in cellular permeability produced by a toxin.

Recent studies on _Corynebacterium_ spp. indicate that they produce phytotoxic polysaccharides in culture media and in the plant. Spencer and Gorin (39) reported that _C. insidiosum_ and _C. sepedonicum_ (Speckerman and Kotthoff) Skaptason and Burkholder, produced viscous polysaccharide solutions in culture media. Partial purification showed that the polysaccharide isolated from _C. insidiosum_ induced wilt in alfalfa cuttings at a concentration of 0.002%. They detected fucose, galactose, and glucose residues in the acid hydrolyzed polysaccharides and offered presumptive evidence that these polysaccharides were present in infected alfalfa and potato plants by demonstration of the rare sugar L-fucose in them. They postulated that the mechanism of action was plugging of xylem vessels. The acidic nature of these polysaccharides was ascribed to 4,6-0-(1'-carboxyethylidene)-D-galactose (16). Patino-Mendez (32) isolated a polysaccharide from a culture of C. michiganense (Erw. Smith) Jensen, and detected fucose, galactose, glucose, mannose, rhamnose and an unidentified compound from the acid hydrolysate of the polysaccharide. He also found that a 0.01% solution of toxin caused wilting in tomato cuttings and postulated plugging as the mechanism of action. In both cases (32, 39) no attempt was made to characterize the toxins other than through their sugar components. Little attempt was made to study their physical properties, to demonstrate homogeneity, or to study their biological parameters. The complete physical, chemical, and biological characterization of these toxins was needed. Strobel (41, 43) reported that the toxin isolated from C. sepedonicum was glycopeptide in nature and possessed 3 sugars (glucose, mannose, L-fucose), 2 unidentified reducing compounds, an unidentified non-reducing compound, 9 amino acids, and 1 organic acid (2-keto-3-deoxygluconic acid). The purified toxin had a molecular weight of 21,400, was highly branched, and had an empirical formula of $C_{48}H_{96}O_{48}N$. He showed that the toxin lost biological activity after acid hydrolysis in 0.5N sulfuric acid, and he demonstrated the presence of the toxin in infected potato plants by a number of methods including infrared and ultraviolet spectral analysis, X-ray crystallography, and high voltage paper and disc gel electrophoresis experiments. Strobel and Hess (42) proposed an alternate theory to mechanical plugging by demonstrating that membranes were being damaged by the toxin.

Johnson and Strobel (25) established the active site of the glycopeptide as the carboxyl group of the organic acid. Rai and Strobel (34, 35) reported the isolation of physiologically active compounds from C. michiganense. They reported that C. michiganense produces 3 biologically active compounds. These toxins also contained the sugar residues glucose, galactose, mannose, and fucose, and 10 amino acid residues. They postulated that the mechanism of action of these glycopeptides was not plugging but membrane damage. They also demonstrated the presence of the toxins in infected plants by serological methods.

The purpose of this thesis is fourfold: (a) to define the homogeneity of the toxic preparation produced by C. insidiosum, (b) to clarify which chemical residues constitute the purified toxin, (c) to measure the physical properties of the toxin, and (d) to determine and measure various biological parameters of the toxin.

MATERIALS AND METHODS

Culturing: A culture of *C. insidiosum* (courtesy of F. I. Frosheiser, University of Minnesota) was maintained on a medium containing 1.5% glucose, 2.0% agar, 1.0% yeast extract dialysate, and 0.5% calcium carbonate (43). The culture was streaked and taxonomically identified as *C. insidiosum* (6). The organism produced wilt symptoms when inoculated by stem wounding into a 2 year old alfalfa plant.

Preparation and purification of toxin: The organism was grown on 250 ml of liquid medium in 1 liter flasks. The culture was aerated on a Psycrotherm Incubator Shaker at 20 rpm at 20° for 4 days. The toxin was isolated in a manner similar to that employed by Spencer and Gorin (15, 39), Strobel (41), and Rai and Strobel (34, 35). The culture was centrifuged at 20,000 xg for 10 minutes and the pellet discarded. The supernatant liquid was treated with 3 volumes of acetone (-15°) and the resulting precipitate pelleted by centrifugation at 10,000 xg for 10 minutes. The pellet was dissolved in 100 ml of distilled water and passed through a column of Dowex 1 (formate form), 2.5 X 5.0 cm, 200-400 mesh, rinsed with 50 ml of water, and the effluent passed through a column of Dowex 50 (H^+ form), 2.5 X 5.0 cm, 200-400 mesh, and rinsed with another 50 ml of water. The effluent was collected and fractionated 2 times with ammonium sulfate according to the procedure of Falconer and Taylor (11). Ammonium sulfate (20-35%) precipitated the toxin which was removed by centrifugation at 10,000 x g for 5 minutes and redissolved in 100 ml of distilled water. The toxin was dialyzed against distilled water for 4 days at 4^{o} with many changes of distilled water. The solution of purified toxin was stored at -15^{o} until used.

Optimum toxin production: Aliquots of a growing culture were removed at various time intervals. The turbidity due to growth was measured with the Klett-Summerson Photoelectric Colorimeter, and the amount of toxin production determined with the Beckman Laboratory Carbonaceous Analyzer. Toxin solution (20 µl) was injected into the instrument and the amount of carbon determined in ppm from a standard curve after calibration of the instrument with acetic acid standards.

Biological assay: The assay procedure used was similar to that employed by Johnson and Strobel (25). Tomato seedlings (Lycopersicon esculentum Mill.) cultivar Earliana and alfalfa seedlings (Medicago sativa L.) cultivars Ladak and Orca grown in vermiculite under continuous illumination for 10-14 days were severed at the crown region and placed in small test tubes containing solutions of the toxin buffered with 0.05M phosphate to a final pH of 7.0. The test tubes were mounted in a plexiglass holder surrounded by a transparent plexiglass box. The top of the box served as a reservoir for water to prevent heating by an artificial light source (100W bulb and a circular fluorescent bulb) placed above it. After given time intervals the cotyledons were removed with a sharp razor blade and the strength of each stem was determined using the wilt-o-meter. This instrument applies a steadily increasing force against a stem until it is no longer able to maintain an erect position. The degree of wilting in any given treatment was the average of three readings obtained from each of 5 stems.

Radioactive methods: *C. insidiosum* was grown on the standard liquid medium containing 25µC of D-galactose-1-^{14}C (specific activity 3.0 mC/mM). The radioactivity in the toxin was determined in the liquid scintillation spectrometer (Model 6804 Nuclear Chicago). The toxin solution (10-20 µl) was placed in a vial along with 1.5 ml of absolute methanol and 13.5 ml of scintillation solution containing 4.0 g of 2,5-diphenyl-oxazole and 100 mg of p-bis-2(5-phenyloxazolyl)-benzene per liter of toluene. The channels ratio method was used to correct for quenching. Autoradiography was performed using Kodak No-screen X-ray film, and electrophoretograms were scanned with a Packard Radiochromatogram Scanner (Model 385). Scanning was performed at 0.5 cm/min with a colliminator width of 5.0 mm, a time constant of 10, a linear range of 300, and a gas flow rate of 350 cc/min.

Molecular exclusion chromatography: The toxin was taken to dryness and 1-5 mg dissolved in 1 ml of 0.05M Tris buffer at pH 7.0 in 40% sucrose. The sample was then fractionated with the buffer through columns of Sepharose 4B or Sepharose 2B. Fractions (1.5 ml) were collected with the aid of a drop counter, and radioactivity and total carbon determined in each fraction. Attempts to dissociate labelled toxin into subunits with 30% pyridine (36) or 8M urea followed by dialysis against distilled water for 48 hours was also followed by fractionation on Sepharose 2B.

Electrophoresis: High voltage paper electrophoresis was conducted in a manner similar to that employed by Strobel (42). One-hundred µg of toxin dissolved in 20 µl of water was distributed over 25 cm in the center of a 34 X 40 cm piece of Whatman #1 filter paper. The paper was pressed between a lower water-cooled plate and an upper surface covered by a flexible plexiglass plate. Pressure was exerted to the upper plate by a water filled rubber bag with a pressure of 6 pounds per square inch. The temperature of the water in the bag was 4^{o}. The molarity and pH of the buffers used were varied in these experiments. All experiments were conducted with a 22.5 v/cm potential for 2 hours. The toxin was detected by the methods of Trevelyan (45), with 0.3% ninhydrin in ethanol, for radioactivity with the chromatogram scanner, and visually for the chromophore.

Disc gel electrophoresis was performed in 5.0% polyacrylamide gels with glycine-Tris buffer, pH 8.8. Fifty µg of labelled sample in 40% sucrose was subjected to 2.5 ma in each acrylamide tube (8). After electrophoresis for 30 minutes, the toxin was detected with aniline blue black for proteins, Schiff's base reagent for carbohydrates (33), for the chromophore, and by autoradiography after drying the gel according to the procedure of Herrick and Lawrence (20). Gels developed by each technique were scanned on a Joyce Chromoscan Densitometer.

Analytical ultracentrifugation: The sedimentation coefficient of the toxin was determined with a Spinco Model E analytical ultracentrifuge using the schleiren optical system on 1.0, 2.6, and 5.0 mg/ml solutions of toxin. Centrifugation was performed at 64,000 rpm and photographs were taken at 32 min intervals with a bar angle of 50°. The diffusion coefficient was determined by creating a synthetic boundary at 8,000 rpm with solutions of the same concentrations. The partial specific volume of the toxic polysaccharide was assumed to be 0.65 (14). The sedimentation coefficient, diffusion coefficient, and molecular weight calculations were calculated by the method of Schachman (38).

Light scattering: The molecular weight of the toxin was determined with the Brice Pheonix Universal Scattering Photometer (Series 2000) using light with a wavelength of 4358Å on a series of 6 solutions ranging from 0.035% - 0.090%. Calculations were based on those of Anacker (2). Double extrapolations to zero angle and zero concentration from the resulting Zimm plot yielded the molecular weight.

Specific viscosity: A Cannon-Ubbelohde semi-micro viscometer, Model 75 (Cannon Instrument Co.), was used to determine the specific viscosity at concentrations of 2.6, 1.3, 0.87, 0.65, 0.52, 0.43, and 0.22 mg/ml solutions. The intrinsic viscosity was the intercept of the graph of specific viscosity/concentration as a function of concentration (38).

Elemental analysis: Carbon, hydrogen, and oxygen were determined by Schwarzkopf Microanalytical Laboratory, Woodside, New York; nitrogen was determined by the microkjeldahl technique. From the percentages of these the empirical formula was calculated.

Chromophore: Five ml of 0.1% solution of toxin was treated with 0.01M ethylenediamine tetraacetic acid (EDTA) at 50^{o} for 15 minutes. The toxin and apotoxin were then dialyzed against distilled water for 48 hours after which the ultraviolet and visible spectrum (240-750 mμ) were measured on a Cary Split-beam spectrophotometer using a 3 ml cuvette with a 1 cm light path.

A 0.1% solution was assayed to determine the presence of metal ions which were measured on a Jarell-Ash atomic absorption flame emission spectrophotometer by removing the ion with EDTA and then separating the EDTA-metal-ion complex from the toxin by dialysis.

Acid hydrolysis: Twenty ml of 0.5N sulfuric acid was added to a 50 ml flask containing 50 mg of toxin. The toxin was refluxed for 8-12 hours. The mixture was cooled, diluted with 100 ml of distilled water, and neutralized with excess barium carbonate. The precipitate was removed by centrifugation and the supernatant liquid passed through a column of Dowex 50 (H^+ form), 0.5 X 2.0 cm, 200-400 mesh, followed by a 10 ml rinse of water and passage through a column of Dowex 1 (OH^- form), 0.5 X 2.0 cm, 200-400 mesh. The Dowex 1 column was rinsed with 10 ml of water and the effluent dried under vacuum desiccation over P_2O_5. This was considered the neutral fraction. The organic acid fraction was obtained by eluting the Dowex 1 column with 10 ml of 6N formic acid. The eluant was dried and stored in an evacuated desiccator over P_2O_5.

The procedure used for amino acid hydrolysis was that of Moore and Stein (30). Toxin (5-10 mg) or peptide (0.25 mg) were placed in constant boiling hydrochloric acid, a crystal of phenol was added, and the tube sealed under vacuum. The tube was heated for 20 hours at 110°. Upon cooling the contents of the tube were dried by flash evaporation at 50° and stored under vacuum desiccation over sodium hydroxide pellets. This preparation was taken up in 5 ml of distilled water and passed through Dowex 50 (H^+ form), 0.5 X 1.0 cm, 200-400 mesh. A 5 ml distilled water rinse followed, and the amino acids were then eluted with 6N hydrochloric acid and dried as above.

Chromatography: Separation and identification of sugar residues was done according to the method of Albersheim, et al. (1). The sugars were reduced to their alditols with sodium borohydride in 1N ammonium hydroxide. The reaction was stopped by the addition of a slight excess of glacial acetic acid and borate removed by 5 methanol evaporations. Acetic anhydride (1 ml) was added, the tubes containing the sugar alcohols sealed, and heated for 3 hours at 121°. The resultant alditol acetates were identified and their amounts estimated by gas-liquid chromatography. The best separation was attained using Gas Chrome P (100-120 mesh) coated with 0.2% poly(ethyleneglycol succinate), 0.2% of poly(ethyleneglycol adipate) and 0.4% of silicone XF 1150. The F and M gas chromatograph electrometer was set at range 100 and attenuation 4. A 6 foot column was used which was heated to 120° for 10 minutes after injection and then raised 1° per minute to 190°. The detector temperature was 250°, and the carrier gas flow rate was 30-50 cc per minute. The amount of each sugar was determined by a comparison with standard curves established with authentic sugars.

Separation of organic acid residues was done by one-dimensional, descending, paper chromatography, on Whatman #1 filter paper in the following solvent systems: (A) 1-butanol:acetic acid:water (4:1:5) v/v, (B) 1-butanol:pyridine:0.1N hydrochloric acid (5:3:2) v/v, (C) ethyl acetate:acetic acid:water (3:1:3) v/v, (D) 2-butanone:acetic acid:water (8:1:1) v/v, (E) 80% phenol v/v, and (F) 1-propanol: cineole:formic acid (5:5:2) v/v.

Identification and quantification of amino acid residues was done on a Beckman automatic amino acid analyzer.

Peptide: The β-elimination procedure described by Anderson, et al. (3) and Tanaka and Pigman (44) to remove peptides from the glycoprotein was used. Toxin (40 mg) was placed in 20 ml of 0.5N sodium hydroxide and incubated for 216 hours at 4° in 0.3M sodium borohydride. The sodium borohydride reaction was stopped by the addition of a slight excess of acetic acid, and borate removed as methyl borate by 5 methanol evaporations. The solution was neutralized with sodium hydroxide and passed through a column of Dowex 50 (H^+ form), 1.0 X 5.0 cm, 200-400 mesh. The retained peptide was then eluted with 6N hydrochloric acid and dried over sodium hydroxide.

Peptide (1 mg) was spotted on a 34 X 40 cm piece of Whatman #1 filter paper and subjected to electrophoresis for 3 hours with 12.5 v/cm potential across the paper in formic acid (0.54M) acetic acid

(1.11M) buffer, pH 1.85 (5). This same electrophoretogram was dried and chromatographed in solvent (A) for 10 hours in the other dimension. The peptide was detected with a modified o-tolidine procedure. The electrophoretogram was passed through acetone:ethanol (1:1) solution and while still moist suspended over a solution of 1N hydrochloric acid and 0.5N potassium permanganate for 5 minutes on each side. The chromatogram was passed through a solution of 0.5M potassium iodide mixed 1:1 with saturated o-tolidine in 2N acetic acid.

$\underline{NH_2}$-Terminal amino acids: The dansyl technique, described by Gray and Hartley (18) was used to determine the NH_2-terminal amino acid in the peptide. One mg of the peptide and 25 mg of sodium bicarbonate were dissolved in 0.7 ml of water and the solution adjusted to pH 8.2. One-tenth ml of a solution containing 1 mg of dansyl chloride per ml of acetone was added and the solution was incubated for 12 hours at room temperature. After drying, followed by acid hydrolysis in 1.0 ml of constant boiling hydrochloric acid for 12 hours, the sample was compared against a series of standards by thin-layer chromatography on Absorbosil #5 in (a) benzene:pyridine:acetic acid (16:4:1) v/v, and in (b) toluene:pyridine:ethylene chlorohydrin:0.8N ammonium hydroxide (10:3:6:6) v/v, upper phase. The dansylated amino acids were detected by ultraviolet light.

Electron microscopic study: A 0.1% solution of toxin was frozen in Freon 22 in the Balzars Apparatus (BA 360M) and freeze-etched as described by Moor (29) and Moor and Muhlethaler (28). The replicas were prepared for examination in the electron microscope as described by Hess and Stocks (21) and examined on a Hitachi HS7 electron microscope.

EXPERIMENTAL RESULTS

Purification and biological activity of toxin: Figure 1 is a flow chart illustrating the procedure used to isolate the toxin. The toxin, represented by the vertical axis, was exocellular, precipitated with 3 volumes of cold acetone, and was not retained on Dowex 1 or 50. The effectiveness of each step in removing contaminating materials is shown in Table I. On a specific biological activity basis the purification factor was 5.57X. The culture produced 1.25 g of toxin per liter.

The tomato seedling assay developed by Johnson and Strobel (25) was valid for use with this toxin. Tomato seedlings (Figure 2) wilted to 100 mg stem strength in a 0.25% solution of buffered toxin in 1 hour when measured on the wilt-o-meter, while both cultivars of alfalfa checked wilted to values of 200 mg. The resistant cultivar of alfalfa (Ladak) wilted to the same degree as the susceptible cultivar (Orca) which implied resistance to the disease is apparently not related to the toxin.

Wilt was dependent on concentration (Figure 3) with measurable wilt occurring in a 0.02% (4×10^{-8}M) solution of toxin and maximal wilt in 0.15% (3×10^{-7}M) solution of toxin in 1 hour. Wilt of the plant cuttings was a function of time and also concentration up to 0.25% toxin solutions (Figure 4).

The toxin still contained contaminating materials after passage

Figure 1. Purification procedure for the toxin produced by C. insidiosum. The toxin was isolated according to the procedure shown. Location of biological activity was determined using the tomato seedling assay. The toxin is exocellular, precipitates with acetone, is not retained on Dowex 1 or 50, and precipitates in the 25-35% fractions with ammonium sulfate.

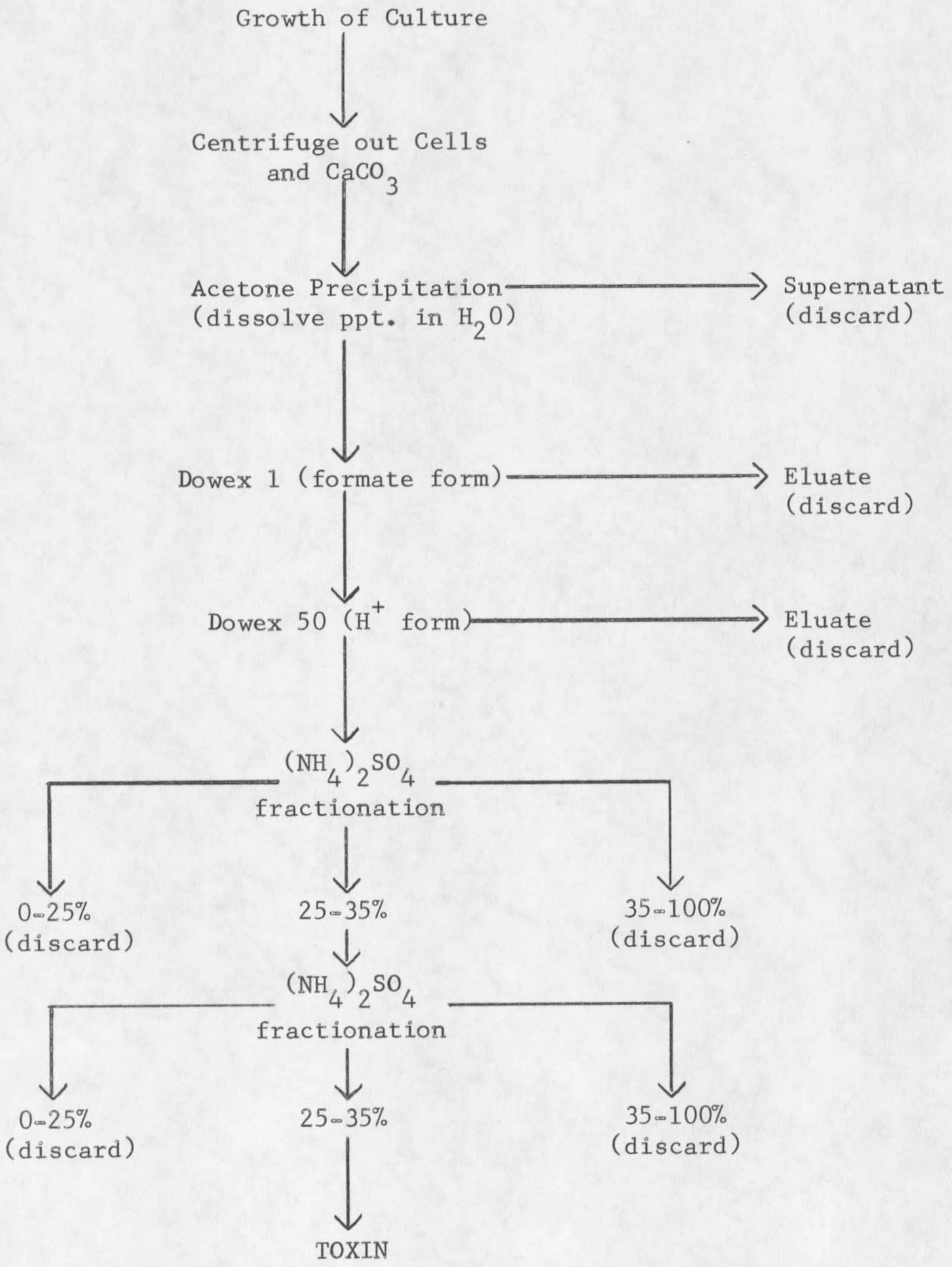
Growth of Culture
Centrifuge out Cells and $CaCO_3$
Acetone Precipitation (dissolve ppt. in H_2O)
Supernatant (discard)
Dowex 1 (formate form)
Eluate (discard)
Dowex 50 (H^+ form)
Eluate (discard)
$(NH_4)_2SO_4$ fractionation
0-25% (discard)
25-35%
35-100% (discard)
$(NH_4)_2SO_4$ fractionation
0-25% (discard)
25-35%
35-100% (discard)
TOXIN

Table I

Effectiveness of each Step in the Purification Scheme in the Isolation of the Toxic Glycopeptide

Step in purification[a]	Dry weight	Specific Biological Activity[b]	Purification Factors
	g	mg	
Crude culture supt.	6.78	418	1.00
Acetone precipitate	2.62	285	1.47
Dowex 1 treatment	2.17	238	1.76
Dowex 50 treatment	1.93	219	1.91
1st $(NH_4)_2SO_4$ ppt.	1.39	127	3.29
2nd $(NH_4)_2SO_4$ ppt.	1.25	75	5.57

[a] For each step in the purification procedure, the total dry weight, the specific biological activity, and the purification factor based on specific biological activity were determined. The data are based on 1 liter of crude culture supernatant liquid as the starting material.

[b] Defined as the amount of strength of a tomato hypocotyl remaining after 1 hour treatment in a 1 mg/ml solution of each preparation as measured by a wilt-o-meter. Buffer controls had a stem strength of 523 mg.