A Comparison of the Chlorosis-Inducing Toxin from Pseudomonas coronafaciens with Wildfire Toxin from Pseudomonas tabaci

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ABSTRACT

We have tentatively concluded that the chlorosis-inducing toxins from *Pseudomonas tabaci* and *P. coronafaciens* are identical or at least very similar. They behaved identically in a number of chromatographic systems. Acid hydrolysis of either toxin yielded tabtoxinine, threonine, and serine in a 1:1:0.1 molar ratio to the toxin. In 0.4 M $NaHCO_3$, the toxins are converted stepwise into two alkali inactivation products. Acid hydrolysis of the first product yields the same compounds as do the toxins, whereas tabtoxinine was the only ninhydrin-positive compound found in hydrolysates from the second product. Lactic acid was not detected as a component of the toxins. Phytopathology 60:360-364.

A number of phytopathogenic *Pseudomonas* spp. cause leaf symptoms characterized by a light yellow halo that surrounds a restricted, necrotic infection site. Woolley, Braun and coworkers (2, 16, 18) isolated and chemically characterized an exotoxin from culture filtrates of *P. tabaci* which reproduced the chlorotic symptom on tobacco. Evidence has also been presented for the similar involvement of chlorosis-inducing toxins in the halo blight diseases of oat, bean, soybean, and tomato (6).

Since in most respects, other than host range, the halo-blight Pseudomonads are indistinguishable (9), it seems likely that the toxins elaborated by these bacteria may be chemically related. This has been suggested for the toxins from *P. phaseolicola* and *P. tabaci* (10). However, we found that the toxins from five *Pseudomonas* spp. fell into two classes (13). The toxin in culture filtrates from *P. coronafaciens* and *P. tabaci* is heat labile, while that from *P. phaseolicola, P. glycinea* and *P. tomato* is heat stable and produces symptoms differing from those of the first class. Culture filtrates from bacteria in the second, but not the first class, also induce ornithine accumulation in bean. However, whether this is due solely to the toxin has not been ascertained.

Our initial objective in studying this group of bacteria was to isolate the chlorosis-inducing toxin from culture filtrates of *P. coronafaciens,* and to compare its properties with those of the wildfire toxin from *P. tabaci*. A preliminary report has been presented (12).

MATERIALS AND METHODS.—Isolates of *P. tabaci* (Wolf & Foster) Stevens and *P. coronafaciens* (Elliott) Stevens were obtained in part from the Department of Bacteriology, University of California, Davis (ICPB), the National Culture Collection of Plant Pathogenic Bacteria, Harpenden, England (NCPPB) and the American Type Culture Collection (ATCC). They were maintained on nutrient agar and in sterile water. Initially, 19 isolates of *P. tabaci* and 32 isolates of *P. coronafaciens* were screened for pathogenicity and toxin production using oat, *Avena sativa* L. 'Andrew'; tobacco, *Nicotiana tabacum* L. 'Bottom Special'; and bean, *Phaseolus vulgaris* L. 'Top Crop'. For pathogenicity tests, a 5-μliter droplet of a cell suspension (2 to 6×10^6 cells/ml) washed twice with distilled water was placed on a leaf, and the leaf lightly pricked under the droplet with a sterile needle to introduce the bacteria into the leaf. Isolates were screened for toxigenicity by using cell-free culture filtrates as described earlier (4). All lesions were measured 4 days after treatment, and the plants were incubated in a 24-C growth chamber held at 2,200 ft-c for 16 hr.

Semiquantitative determinations of toxin concentration in culture filtrates and in purified preparations were made by applying different dilutions of sterile, neutralized solutions on fully expanded primary leaves of bean. Six 5-μliter droplets of each dilution were placed on each of three leaves. L-Methionine-DL-sulfoximine (L-MSO) was used as a standard. After treatment, the plants were incubated as above, and the lesion size measured to the nearest mm 3 days later. The results are expressed as an average of the 18 lesions. A difference of 0.5 mm in average halo size was significant at the 1% confidence level. One unit of toxin activity was defined as that amount which would produce a 2-mm diam halo/5-μliter application droplet; this is equivalent to 0.08 μg L-MSO. Two units of activity produced a 3.9 mm lesion, while 4 units produced a 5.5-mm lesion.

Although nutrient broth as well as a number of other media, including those used by Rudolph (10), Garber (5), and Hoitink (8) supported growth of all isolates, toxin production was always significantly higher in the synthetic medium of Woolley et al. (16). Therefore, for production and purification studies, isolates of the two species were grown simultaneously using this medium. For large-scale toxin production, 15-liter fermentation tanks were inoculated with 500 ml of a log-phase culture, and the tanks vigorously aerated (14 liter/min) and stirred (365 rpm) at 24 C for 5 days.

After removal of the cells by centrifugation, the culture filtrates were adjusted to pH 4.5 with HCl, treated with charcoal (Norit A), concentrated under vacuum at 20 C, and methanol was added as described by Woolley et al. (16). After evaporating the methanol and dissolving the residue in 0.2 M citrate buffer, pH 2.2, 5-ml aliquots, containing about 5×10^3 units of activity, were applied to a Beckman PA-28 ion-exchange resin column. The toxin was eluted using a modified elution schedule (3) in which the detergent

(BRIJ), thiodiglycol, and caprylic acid had been omitted from the buffers. After concentrating, the fractions containing toxic activity were desalted using a column of Sephadex G-10 (75 × 1.5 cm). Throughout the purification procedure, the preparations were frozen when possible.

In order to detect inactivation products of the toxin, active fractions eluted from the PA-28 column were rechromatographed 24 hr after the following treatments: (i) freezing immediately upon elution; (ii) incubating 2 hr at room temperature in the elution buffer (0.2 M sodium citrate, pH 3.12), then freezing; and (iii) heating (100 C for 20 min), then freezing. For comparison with the data of Woolley et al. (16, 18), samples of the purified toxins were treated with 0.4 M $NaHCO_3$ at room temperature for 3 hr and with 1 N NaOH at 100 C for 1 hr, then assayed for biological activity and chromatographed.

Paper and thin-layer Silica Gel G chromatography were used to compare the toxins and their inactivation products. For comparison of R_F values with those reported for the wildfire toxin, the paper chromatographic procedures of Woolley et al. (16, 17, 18) were also employed. Strips of the developed chromatograms upon which the purified toxins from *P. coronafaciens* and *P. tabaci* had been applied were sprayed with ninhydrin while adjacent strips were cut into 5-mm sections. These sections were eluted with water, and the eluates assayed on tobacco for toxic activity.

The retention times of the toxins and their inactivation products were also determined by making two consecutive analyses using the PA-28 ion-exchange resin column. In the first analysis, toxic activity was determined by assaying 5-μliter samples from 0.83-ml fractions on tobacco. In the second analysis, reference amino acids were added to the preparations, and the eluate from the column was analyzed colorimetrically with ninhydrin. Quantities of the toxins and their inactivation and hydrolysis products are expressed in μmoles of ninhydrin reactivity (glycine equivalents) calculated from the chromatograms.

The purified toxins were also acid hydrolyzed (6 N HCl at 110 C for 12 hr). After hydrolysis and removal of the acid under vacuum, the products were separated by preparative ion-exchange chromatography using Dowex 50 × 8 (7), or chromatographed by using the PA-28 column. The amino acid tentatively identified as tabtoxinine was crystallized by the cautious addition of ethanol to an aqueous solution. The long, needle-shaped crystals which formed were recrystallized by the addition of acetone to an aqueous solution.

L(+)-lactic acid was determined quantitatively in toxin hydrolysates using lactic dehydrogenase (2). Two-tenths-μmole samples (glycine equivalents) of the purified toxins in 0.05 ml of 1 N NaOH were heated for 1 hr (18) and then neutralized with 1 N HCl. To other samples of the toxins, a 0.1 μmole of L(+)-lactic acid was added before hydrolysis to determine per cent recovery.

RESULTS.—For all isolates of *P. coronafaciens* and *P. tabaci* that were both pathogenic and toxigenic, there was a close correlation between halo size, produced by the culture filtrate, and halo size resulting from inoculation with the washed bacteria. On the basis of results from this test, three isolates of each species were selected for all subsequent studies (Table 1).

Final toxin titer in the 15-liter fermentation tanks varied from 32 to 128 × 10^5 units/liter, depending on the isolate used. Under the prescribed cultural conditions, toxin titer reached a maximum at 4 days, remained constant for an additional 2 to 3 days, and then declined.

More than 50% of the initial toxic activity was lost during the preliminary steps of purification (Table 2). Because of the small samples of pure toxins prepared (< 2 mg) and their deliquescent nature, the specific activities (units/mg) could not be determined with great accuracy. However, they were close to the value reported by Woolley et al. (16) for the wildfire toxin, i.e., 1 unit/.05 μg. The units/μmole ninhydrin reactivity, determined from ion-exchange chromatography of the toxins, were identical.

When blue dextran 2,000 and NaCl were added to purified toxin preparations and the mixture applied to

TABLE 1. Pathogenicity and toxin production by selected isolates of *P. tabaci* and *P. coronafaciens*

Isolate	Source[a]	Lesion size[b] Inoculation[c] Oat	Tobacco	Toxin assay[d] Bean
		mm	*mm*	*mm*
P. tabaci				
Pt-3	ICPB		11	16
Pt-5	ICPB		10	13
Pt-13	ICPB		10	15
P. coronafaciens				
Pc-17	ICPB	5 × 9		12
1357	NCPPB	4 × 9		10
19608	ATCC	5 × 10		13

[a] ICPB = Dept. of Bacteriology, Univ. Calif., Davis; NCPPB, National Culture Collection of Plant Pathogenic Bacteria, Harpenden, England; ATCC, American Type Culture Collection.
[b] Average of 3 lesions.
[c] 5-μliter droplet of washed inoculum adjusted to 0.1 OD at 660 nm.
[d] 5-μliter droplet of sterilized culture filtrate.

TABLE 2. Purification of the toxin from culture filtrates of *P. tabaci* (isolate Pt-4) and *P. coronafaciens* (isolate Pc-17)

Purification step	Total units × 10^{-5}/liter filtrate		Specific activity units/mg	dry wt
1. Crude culture filtrate	58	120	4.1	6.7
2. Norit A treated	36	53	22	31
3. Methanol extract	35	47	160	230
4. Ion-exchange column eluate	35	40	[a]	[a]
5. Desalted toxin[b]	18	12	34,000	41,000

[a] Specific activity on a dry wt basis not determined. The units of activity/μmole ninhydrin reactivity (glycine equivalents) were 9,600 for both toxins.
[b] Toxin contained traces of inactivation products.

the Sephadex G-10 column, both toxins were eluted at 1.1 times the void volume (V_0), while NaCl eluted at 1.4 V_0. Since the exclusion limit of Sephadex G-10 is approximately 700, it follows that the toxins have a molecular wt of less than 700.

Paper chromatographic comparisons of the toxins from *P. coronafaciens* and *P. tabaci* using methanol extracts from all six isolates showed that the two species produced toxins with identical R_F values; using *n*-propanol:water or phenol:water, the toxins had R_F values of 0.26 or 0.28, respectively (Table 3). Several other ninhydrin-positive spots were always present on the chromatograms. However, they had no toxigenic activity.

The retention time for the toxin from either *P. coronafaciens* or *P. tabaci* on the PA-28 ion-exchange resin column was 163 min (Fig. 1). Cochromatography of equal amounts of toxin from methanol extracts of *P. coronafaciens* and *P. tabaci* cultures resulted in a single symmetrical peak of biological activity and ninhydrin reactivity. Even after the toxins had been purified by collection from the column, and desalted by means of gel filtration, the purified toxin preparations always contained traces of two other ninhydrin-reactive compounds (Fig. 2-A, C). These two compounds were later shown to be inactivation products of the toxins. On paper chromatograms only the toxin spots could be observed, unless more than 5 μg of the preparations had been applied. R_F values of the purified toxin and its inactivation products in 7 different solvent systems are listed in Table 3.

Acid hydrolysis of the purified toxins resulted in the appearance of three new ninhydrin-positive peaks on chromatograms with retention times of 85, 95, and 225 min (Fig. 2-B, D). Two of these hydrolysis products had retention times identical to those of threonine and serine. They were also inseparable from the authentic amino acids in all paper, ion-exchange, and thin-layer cochromatography tests (Table 3).

The third hydrolysis product, which had a retention time of 225 min, was tentatively identified as tabtoxinine (17). This compound reacted with ninhydrin to give a colored complex with an unusually high ratio of 440:570 nm absorbancies. Among the amino acids commonly found in protein hydrolysates, only glutamic acid and cystine have a similar absorbancy ratio. However, these two amino acids can be separated from the unknown by using either paper or ion-exchange chromatography. On paper chromatograms, the third hydrolysis product had R_F values very near or identical to those reported for tabtoxinine (Table 3). Cochromatography of tabtoxinine isolated from *P. coronafaciens* toxin hydrolysates with authentic tabtoxinine on an amino acid analyzer at Rockefeller University produced a single symmetrical peak. In addition, our compound was oxidized by periodate exactly like authentic tabtoxinine (Stewart, W., *personal communication*). The previous report that tabtoxinine isolated from *P. coronafaciens* or *P. tabaci* toxin did not react with periodate (12) was based on the results of a micro-periodate oxidation test described by Aldeberg (1), in which the concentration of periodate employed is not sufficient to oxidize tabtoxinine.

The hydrolysis products identified as threonine and tabtoxinine were produced within 85-100% of a mole-for-mole ratio to the toxin, regardless of the bacterial species used to produce the toxin. A typical analysis yielded, per mole of toxin hydrolyzed (glycine equivalents), 0.98 mole of threonine, 0.05 mole of serine, and 0.93 mole of tabtoxinine.

If the toxin-containing fractions were immediately frozen upon elution from the PA-28 column, no other compound could be detected upon rechromatography 24 hr later; and there was no loss in biological activity. If, however, the fractions were incubated for 2 hr at room temperature prior to freezing, traces of two inactivation products appeared with retention times of 195 and 282 min.

Heating the toxins at 100 C for 20 min in the elution buffer resulted in a loss of biological activity of about 50%, a decrease in the area of the toxin peak, and an increase in the quantities of the two inactivation products. When collected from the PA-28 column and desalted, the two inactivation products (labeled "195" and "282" in Table 3) did not separate from each other on paper chromatograms developed in *n*-propanol:water. Both compounds had R_F values in this solvent system similar to that of the "alkali inactivation

TABLE 3. R_F values of the toxins from *P. coronafaciens* and *P. tabaci* and their hydrolysis and inactivation products

	Solvent systems[a]						
	1	2	3	4	5	6	7
Toxins	0.26	0.28		0.02	0.22	0.34	
Tabtoxinine	0.10 (0.08)[b]	0.09 (0.10)	0.21 (0.21)	0.20	0.05	0.08	0.02
"Threonine"[c]	0.38	0.44	0.55	0.14	0.28	0.43	0.25
"Serine"[c]	0.29	0.35	0.42		0.20	0.36	0.22
"195"[d]	0.22	0.47			0.23	0.30	
"282"[d]	0.23	0.48			0.27	0.24	

[a] 1 = *n*-Propanol:water (2:1), Whatman No. 1 filter paper; 2 = phenol:water (4:1), Whatman No. 1; 3 = phenol: ammonia:water (500:3:125), Whatman No. 1; 4 = *n*-butanol:acetic acid:water (4:1:5), Whatman No. 1; 5 = phenol:water (4:1, 20 mg NaCN/100 ml), Silica Gel G; 6 = *n*-propanol:water (64:36), Silica Gel G; 7 = *n*-butanol:acetic acid:water (6:2:2), Silica Gel G.

[b] Values reported by Woolley et al. (17).

[c] Identical values were obtained for the authentic amino acid.

[d] Elution time in min from a PA-28 ion-exchange resin column.

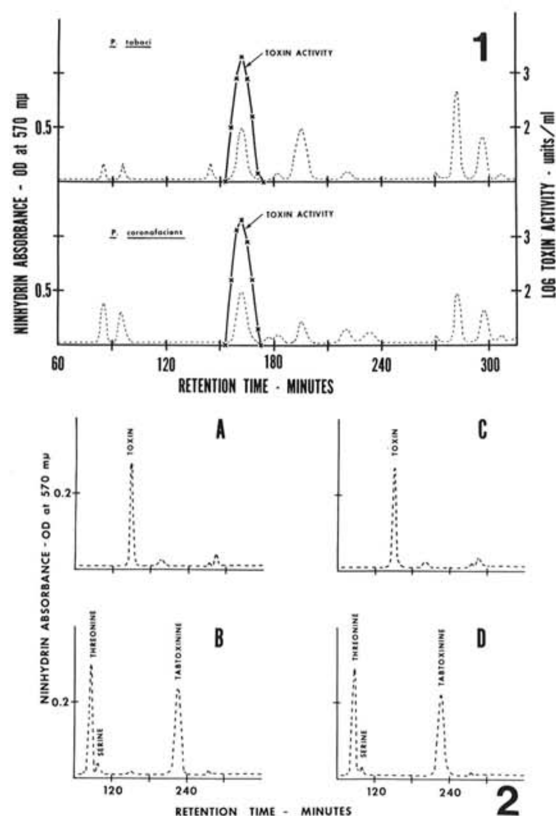

Fig. 1-2. 1) Chromatograms of amino acid analyses of toxin preparations from cultures of *Pseudomonas tabaci* and *P. coronafaciens*. Toxin activity in fractions collected from the column was determined in consecutive analyses by bioassay. **2)** Chromatograms from amino acid analyses of purified toxins and their acid hydrolysis products. The small peak at 270 min represents the buffer change. **A)** Purified toxin from *P. tabaci*. **B)** Acid hydrolysis products of toxin from *P. tabaci*. **C)** Purified toxin from *P. coronafaciens*. **D)** Acid hydrolysis products of toxin from *P. coronafaciens*.

product" of Woolley et al. (18). However, on thin-layer chromatograms the two compounds separated into two distinct ninhydrin-reactive spots (Table 3).

Treatment of the toxins with 0.4 M $NaHCO_3$ also produced these same two inactivation products, but loss in activity from this treatment was less than 50% as determined by both bioassay and chromatography. Both inactivation products lacked chlorosis-inducing activity, even when concentrations of up to 0.2 μmole of either of the purified inactivation products was applied to a tobacco leaf. By comparison, 1×10^{-4} μmoles of the purified toxins produced a 2-mm halo, while 5×10^{-4} μmoles produced a halo of 6 mm.

After the inactivation product which eluted from the PA-28 column at 195 min was heated (100 C for 20 min) in the elution buffer, a second inactivation product, eluting at 282 min, could be detected on the chromatograms. Acid hydrolysis of the inactivation product eluting at 195 min produced the same three amino acids, threonine, serine, and tabtoxinine, in the same molar ratio as found with toxin hydrolysates. The other inactivation product, eluting at 282 min, was stable to heating under the same conditions. Chromatograms of acid hydrolysates of this inactivation product showed only the presence of tabtoxinine, in a mole-for-mole relation to the inactivation product.

Enzymatic assays for L(+)-lactic acid in alkaline hydrolysates of the purified toxins failed to show its presence. When L(+)-lactic acid was added to the toxin preparation before hydrolysis, it was recovered quantitatively, indicating that no products in the toxin preparations or in the hydrolysates were interfering with the assay. Amino acid analyses of alkaline hydrolysates showed that the toxins were completely hydrolyzed by 1 N NaOH to yield the same amino acids as were found in acid hydrolysates.

DISCUSSION.—*P. coronafaciens* synthesizes a toxin which produces chlorotic lesions on oats, tobacco, and bean indistinguishable from those produced by the wildfire toxin or L-MSO. The two toxins were chromatographically identical. Like the wildfire toxin, the oat toxin is unstable under acid or alkaline conditions. The inactivation products of both toxins have the same R_F values on paper or thin-layer chromatograms and identical retention times on ion-exchange chromatography. In addition, their acid hydrolysis products are indistinguishable by chromatographic methods. This evidence strongly suggests that either the toxin synthesized by *P. coronafaciens* is identical to the wildfire toxin or that the two molecules are very similar structurally.

Although we identified tabtoxinine as a component in the acid hydrolysates of both toxins, the other two hydrolysis products, threonine and serine, have not been reported previously as components of the wildfire toxin (18). Threonine and tabtoxinine were detected in a 1:1 molar ratio to the toxin in the acid hydrolysates of the toxin isolated from both bacteria. Tabtoxinine has a molecular wt of 205 (17); threonine has a molecular wt of 119. Since gel filtration experiments showed that the maximum molecular wt of the toxin is less than 700, each mole of toxin probably contains 1 mole of tabtoxinine and 1 mole of threonine.

Serine may have been produced as a result of the hydrolysis of a second compound containing tabtoxinine and serine. Serine was present in hydrolysates of the toxin from both bacterial species in less than a 0.1 mole:mole ratio to the toxin and tabtoxinine. Since the only difference between threonine and serine is a single methyl group, the two compounds, both containing tabtoxinine and differing only with respect to threonine or serine, would be difficult to resolve by means of ion-exchange or solvent partition chromatography. However, the origin of serine in toxin hydrolysates and its significance remain to be determined.

Woolley et al. (16, 18) found that the wildfire toxin was converted to an inactive product when it was incubated in mild alkaline conditions. The toxins produced by *P. coronafaciens* and the isolates of *P. tabaci* we used were also partially inactivated by this treatment, but by means of ion-exchange chromatography we found that two inactivation products were produced by this treatment. However, on paper chromatograms

these two inactivation products had essentially identical R_F values. The failure to detect L(+)-lactic acid in toxin hydrolysates does not rule out the possibility of a different isomer of lactic acid as a component of the toxin, since the enzyme used in this test is specific for the L(+) form of the acid.

In spite of the structural differences between the toxin that we have partially characterized from *P. coronafaciens* and *P. tabaci* and that proposed for the wildfire toxin (16, 17, 18), it seems unlikely that Woolley et al. worked with a different toxin. Two of the three selected isolates of *P. tabaci* used in this investigation were originally isolated and used by Braun. Moreover, the toxin from cultures of *P. coronafaciens* and *P. tabaci* had most of the properties ascribed to the original wildfire toxin. On paper chromatograms the toxin used in this investigation had the same R_F values as those of the wildfire toxin, and released tabtoxinine upon hydrolysis.

Garber & Shaeffer (5), using crude methanol extracts of culture filtrates from five toxigenic *Pseudomonas* spp., found that the toxins all differed in chromatographic behavior. However, the R_F values that they obtained for the toxins from *P. coronafaciens* and *P. tabaci* were quite close; 0.30-0.35 and 0.25-0.30, respectively. Since impurities in the extracts or different toxin concentrations could have influenced the movement of the toxin molecules on paper chromatograms, their results were not conclusive evidence for different toxins. Furthermore, the methanol extracts from cultures of the different bacterial species were not cochromatographed.

A complete structural characterization of the toxin is needed to resolve the differences between the postulated structure of the wildfire toxin (14, 15, 18) and the results obtained in this investigation. Characterization is also necessary to determine definitively whether toxin from *P. coronafaciens* is identical to that produced by *P. tabaci*. If such is the case, a re-examination of the etiological relationship among these and other chlorosis-inducing *Pseudomonas* spp. would appear warranted.

LITERATURE CITED

1. Aldeberg, E. A. Quantitative determination of 1,2-glycols in mixtures. Anal. Chem. 25:1553-1554.
2. Braun, A. C. 1955. A study on the mode of action of the wildfire toxin. Phytopathology 45:659-664.
3. Durbin, R. D., G. F. Pegg, & M. Strmecki. 1967. A modified elution schedule for accelerated amino acid analysis. J. Chromatog. 28:429-431.
4. Durbin, R. D., & S. L. Sinden. 1967. The effect of light on the symptomatology of oat halo blight. Phytopathology 57:1000-1001.
5. Garber, E. D., & S. G. Shaeffer. 1963. Genetic and biochemical characteristics of six phytopathogenic species of *Pseudomonas*. Botan. Gaz. 124:324-328.
6. Goodman, R. N., Z. Kiraly, & M. Zaitlin. 1967. The biochemistry and physiology of infectious plant disease. D. Van Nostrand Co., Inc., Princeton, N. J. 354 p.
7. Hirs, C. H. W., S. Moore, & W. H. Stein. 1952. Isolation of amino acids by chromatography on ion exchange columns; use of volatile buffer. J. Biol. Chem. 195:669-683.
8. Hoitink, H. A. J., R. L. Pelletier, & J. G. Coulson. 1966. Toxemia of halo blight of beans. Phytopathology 56:1062-1065.
9. Lelliott, R. A., Eve Billings, & A. C. Hayward. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29:470-489.
10. Rudolph, K., & M. A. Stahmann. 1966. The accumulation of L-ornithine in halo blight-infected bean plants (*Phaseolus vulgaris* L.) induced by the toxin of the pathogen *Pseudomonas phaseolicola* (Burkh.) Dowson. Phytopathol. Z. 57:29-46.
11. Sigma Tech. Bull. 825-UV, Sigma Chem. Co., St. Louis, Mo. 10 p.
12. Sinden, S. L., & R. D. Durbin. 1968. The identity of the toxin produced by *Pseudomonas coronafaciens* with the wildfire toxin from *P. tabaci*. Phytopathology 58:1067-1068 (Abstr.).
13. Sinden, S. L., & R. D. Durbin. 1969. Some comparisons of chlorosis-inducing pseudomonad toxins. Phytopathology 59:249-250.
14. Stewart, J. M. 1961. α,ε-Diamino-β-hydroxypimelic acid. II. Configuration of the isomers. J. Amer. Chem. Soc. 83:435-439.
15. Stewart, J. M., & D. W. Woolley. 1956. α,ε-Diamino-β-hydroxypimelic acid. I. Synthesis of isomer A. J. Amer. Chem. Soc. 78:5336-5338.
16. Woolley, D. W., R. B. Pringle, & A. C. Braun. 1952. Isolation of the phytopathogenic toxin of *Pseudomonas tabaci*, an antagonist of methionine. J. Biol. Chem. 197:409-417.
17. Woolley, D. W., G. Schaffner, & A. C. Braun. 1952. Isolation and determination of structure of a new amino acid contained within the toxin of *Pseudomonas tabaci*. J. Biol. Chem. 198:807-813.
18. Woolley, D. W., G. Schaffner, & A. C. Braun. 1955. Studies on the structure of the phytopathogenic toxin of *Pseudomonas tabaci*. J. Biol. Chem. 215:485-493.