ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1986, p. 488–495
0066-4804/86/030488-08$02.00/0

Vol. 29, No. 3

Characterization of an Antibiotic Produced by a Strain of *Pseudomonas fluorescens* Inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp.†

SARANGAMAT GURUSIDDAIAH,[1] DAVID M. WELLER,[2]* ANNAPOORNA SARKAR,[1] AND R. JAMES COOK[2]

Bioanalytical Center, Washington State University, Pullman, Washington 99164,[1] and U.S. Department of Agriculture Agricultural Research Service Root Disease and Biological Control Research Unit, Pullman, Washington 99164[2]

Received 9 September 1985/Accepted 12 December 1985

The production, isolation, and characterization of an antibiotic substance from cultures of *Pseudomonas fluorescens* 2-79 (NRRL B-15132) is described. *P. fluorescens* 2-79 originally was isolated from the roots of wheat and is suppressive to the wheat root disease take-all caused by *Gaeumannomyces graminis* var. *tritici*. The antibiotic was isolated from potato glucose broth cultures of strain 2-79 by solvent extraction. It was purified by silica gel column chromatography and was a greenish yellow, needle-shaped crystal with a melting point of 242°C (decomposition). It was soluble in methylene chloride, chloroform, acetone, 2 N sodium hydroxide, and 2 N hydrochloric acid and was insoluble in water, methanol, ethyl acetate, tetrahydrofuran, diethyl ether, carbon tetrachloride, hexane, and petroleum ether. On the basis of UV, infrared, ^{1}H-nuclear magnetic resonance, ^{13}C-nuclear magnetic resonance, mass spectral analysis, and elemental analysis, the structure of the antibiotic is proposed to be a dimer of phenazine carboxylic acid. Lithium aluminum hydride reduction of the antibiotic yielded hydroxymethyl phenazine as a major product which retained most of the biological characteristics of the parent molecule. There were no toxic symptoms when mice received this antibiotic by oral doses up to 464 mg/kg. The antibiotic showed excellent activity against several species of fungi, including the wheat pathogens *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani*, and *Pythium aristosporum*; and it may have a role in suppression of take-all in vivo by strain 2-79.

Pseudomonas fluorescens 2-79 (NRRL B-15132) (12) originally was isolated from the roots of wheat growing in soil from a field where the wheat root disease take-all caused by *Gaeumannomyces graminis* var. *tritici* had spontaneously declined (9). When cells of this bacterium are applied to wheat seed, the bacteria colonize the roots and suppress the pathogen on the roots (12). Strain 2-79 is highly inhibitory to *G. graminis* var. *tritici* in vitro on several media (12), and antibiotic and siderophore production probably are important mechanisms of suppression of take-all in vivo (D. M. Weller, W. J. Howie, and R. J. Cook, Phytopathology 75:1301, 1985). In this report we describe an antimicrobial compound produced by *P. fluorescens* 2-79 that may be involved in suppression of take-all in vivo. This description deals with the production, purification, and characterization of the antibiotic.

MATERIALS AND METHODS

Maintenance of stock culture of *P. fluorescens* 2-79. Strain 2-79 can be grown on most common media including King medium B (7), potato glucose agar, nutrient agar (Difco Laboratories, Detroit, Mich.), and nutrient agar containing 0.5% yeast extract (Difco). The strain can be maintained in a lyophilized state in sealed ampoules at 5°C and in sterile soil in screw-cap tubes at 8°C for a prolonged period with no loss in antibiotic-producing ability.

Cultural conditions for production of the antibiotic. For the production of the antibiotic, strain 2-79 was grown in potato glucose broth. To prepare potato glucose broth, 70 g of unpeeled potatoes (Russet Burbank, Saco, and other cultivars) were homogenized in 500 ml of tap water, and then 20 g of commercial corn sugar (glucose) and 8 ml each of macro- and micronutrient solutions (5) were added. The volume was adjusted to 1 liter with tap water. The broth (200 ml) was placed in 500-ml Erlenmeyer flasks and autoclaved at 121°C for 35 min. After autoclaving, 200 μl of a sterile ferric ammonium citrate stock solution (20 mg/ml of deionized water) was added to each flask. The flasks were inoculated with bacteria from a slant of nutrient broth yeast extract agar (11) and then incubated at 24 to 26°C on a Gyrotory shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 250 rpm for 4 to 5 days.

Antimicrobial assays and mammalian toxicity. Antimicrobial activities of the culture broth and of material extracted from the culture by methylene chloride were determined by the paper disk agar diffusion bioassay method. *Bacillus subtilis*, *Escherichia coli*, *G. graminis* var. *tritici*, and *Saccharomyces* sp. were used as test organisms. The MICs of the purified antibiotic to several species of bacteria were determined by the agar diffusion method, and MICs to fungi were determined by the method of Gurusiddaiah et al. (4). Mammalian toxicity in mice was determined after the antibiotic was administered by oral doses.

Isolation and purification of the antibiotic from shake

TABLE 1. Silica gel thin-layer chromatographic migration of the antibiotic produced by *P. fluorescens* 2-79

Solvent system	R_f
CH_2Cl_2 (100%)	0.145
CH_2Cl_2-ethyl acetate (9:1)	0.418
CH_2Cl_2-acetone (4:1)	0.66
CH_2Cl_2-methanol (9:1)	0.56
$CHCl_3$-acetone (4:1)	0.655

* Corresponding author.

† This is College of Agriculture Research Center Scientific Paper no. SP 7262.

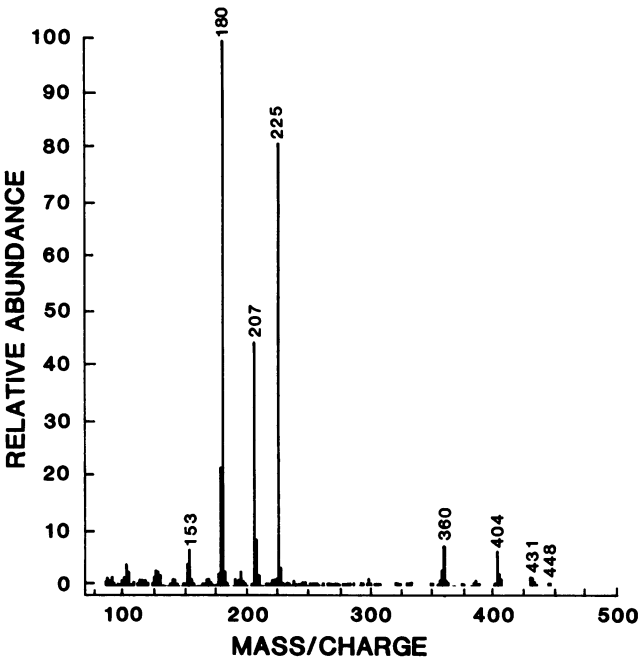


FIG. 1. Mass spectrum of the antibiotic from *P. fluorescens* 2-79.

cultures. After the cultures were incubated for 4 to 5 days, the antibiotic was extracted by adding 100 to 150 ml of methylene chloride to each of 12 flasks (each containing 200 ml of broth culture) and then shaking for 1 to 2 h. The resultant emulsion from several flasks was pooled and then passed through layers of cheesecloth or cotton or both to break the emulsion. After separation of the aqueous and organic solvent phases, the relatively inactive aqueous phase was discarded without further processing, and the active methylene chloride phase was evaporated under reduced pressure. The residue (1.1 g) was then dissolved in a small volume (25 to 30 ml) of methylene chloride, filtered, and evenly adsorbed onto dry silica gel (5 g). After evaporation of the solvent, the charged silica gel was layered over the top of previously packed, dry silica gel in a column (2.5 by 25 cm; silica gel, 30 g). The column was washed exhaustively with methylene chloride (2 liters) to remove some orange-yellow inactive pigments. The main active band (yellow) was selectively eluted from the silica gel column with a mixture of acetone and methylene chloride (1:10 [vol/vol]; 1.5 liters). After the solvent was evaporated, the resulting bright greenish yellow residue was dissolved in 15 to 20 ml of methylene chloride, to which was added 2 to 3 ml of absolute methanol. The solution mixture was warmed on a water bath to evaporate excess solvent or until a few needle-shaped crystals were formed in the container. At that stage the solution was kept overnight at −10°C to crystallize the antibiotic. The fine greenish yellow, needle-shaped crystals (800 to 900 mg) were washed with a few milliliters of methanol and then with petroleum ether and dried in a dessicator.

Alternatively, the antibiotic could be readily crystallized in hexane-methylene chloride (2:1). For this method of crystallization, the antibiotic was first dissolved in a minimum volume of methylene chloride, and then hexane was added until a faint turbidity developed. The solvent mixture was allowed to evaporate slowly at room temperature for 3 to 4 days to obtain long (10 to 15 mm) greenish yellow, needle-shaped crystals.

Homogeneity test of the antibiotic. Homogeneity of the crystallized antibiotic samples was determined by silica gel, thin-layer chromatography (precoated glass plate: silica gel S: 250 F, 250 μm; hard-surfaced analytical layer; obtained from J. T. Baker Chemical Co., Phillipsburg, N.J.) with several solvent systems (Table 1). The chromatograms were viewed under UV light and were then sprayed with vanillin (0.5%) in sulfuric acid-ethanol (4:1 [vol/vol]).

The homogeneity of the antibiotic was also determined by gas-liquid chromatography (Hewlett Packard 5790A chromatograph) using a capillary column (12.5 m by 0.31 mm) coated with methyl silicone. The flow rate of the carrier nitrogen gas was 1 ml/min with programmed temperature.

Spectroscopy of the antibiotic. The UV and visible spectra

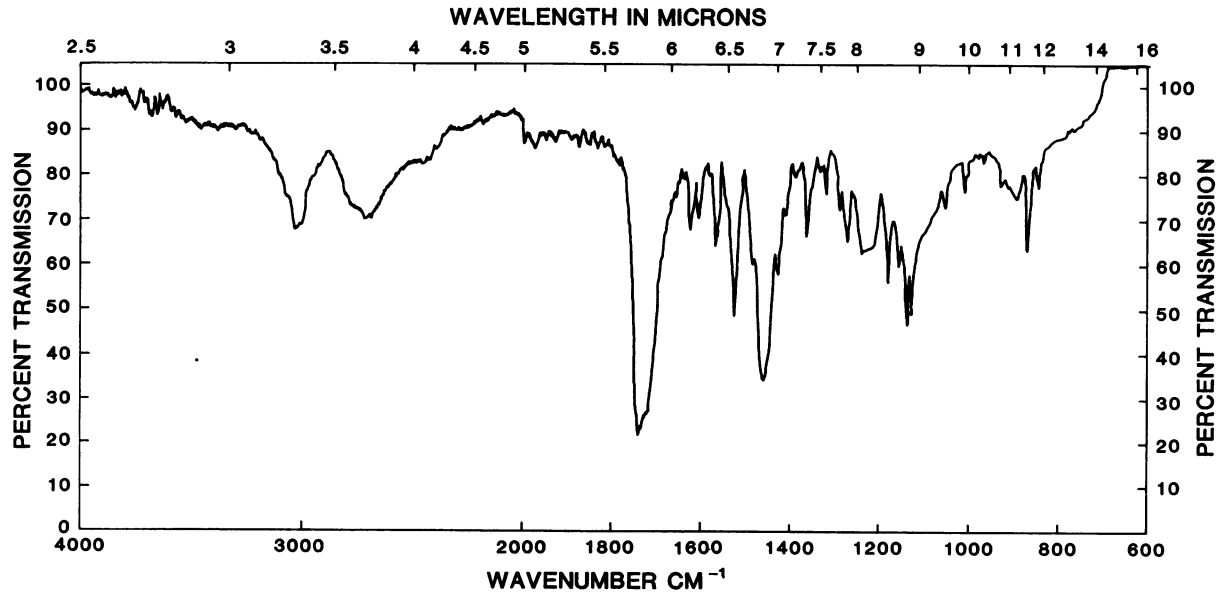


FIG. 2. Infrared spectrum of the antibiotic from *P. fluorescens* 2-79 in $CHCl_3$.

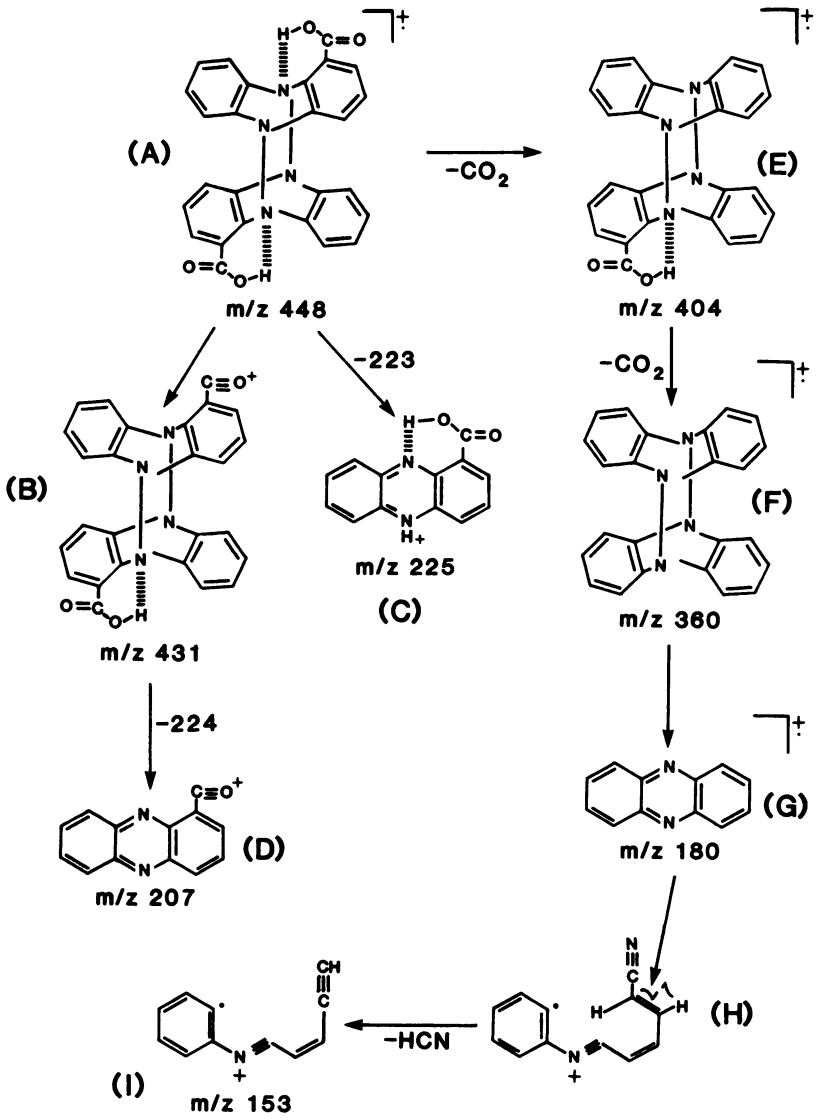


FIG. 3. Assignment of the fragments from the mass spectrum of the antibiotic from *P. fluorescens* 2-79.

of the antibiotic were measured in spectroscopic methylene chloride with a Perkin Elmer spectrophotometer. Infrared spectra of the antibiotic were obtained in chloroform (0.1-mm cell) with a Beckman Acculab 1 infrared spectrophotometer. The proton magnetic resonance spectrum of the parent compound and its derivatives were recorded on a Nicolet magnetic resonance spectrometer model NT-20WB at 200.042 MHz in deuterated chloroform. Mass spectra of the compounds were taken on a VG 7070E gas-liquid spectrometer with argon gas as a carrier. Mass spectra were taken under electron impact, chemical ionization, and fast atom bombardment modes.

Elemental analysis. Elemental analysis was performed in duplicate by M-H-W Laboratories, Phoenix, Ariz. Tests for various halogens, sodium, and other elements in the antibiotic molecule were performed at the Nuclear Radiation Center, Washington State University, by neutron activation analysis.

Reduction of the antibiotic by lithium aluminum hydride. Lithium aluminum hydride (20 mg) was added to 30 ml of tetrahydrofuran and mixed under argon for 15 min at 0°C. To this mixture were added 112 mg of the antibiotic previously suspended in tetrahydrofuran-diethyl ether. The reaction was allowed to continue for about 45 min and was then terminated by the addition of 5 ml of a saturated solution of ammonium chloride. The aqueous layer was extracted three to four times with diethyl ether; concentration of the ether extract resulted in a red gummy substance. On chromatographic analysis by thin-layer chromatography, the gummy material was found to contain at least three major components. One of these was further purified by flash chromatography on silica gel with methylene chloride-diethyl ether (3:1) as the eluting solvent.

RESULTS AND DISCUSSION

Production of the antibiotic. *P. fluorescens* 2-79 produced a greenish-yellow colored antibiotic when grown in potato glucose broth. When ferric ammonium citrate was added to the medium at 100 μg/ml, production of the antibiotic in the broth was enhanced. Surprisingly, the yield of the antibiotic varied between 10 and 700 mg/liter from batch to batch,

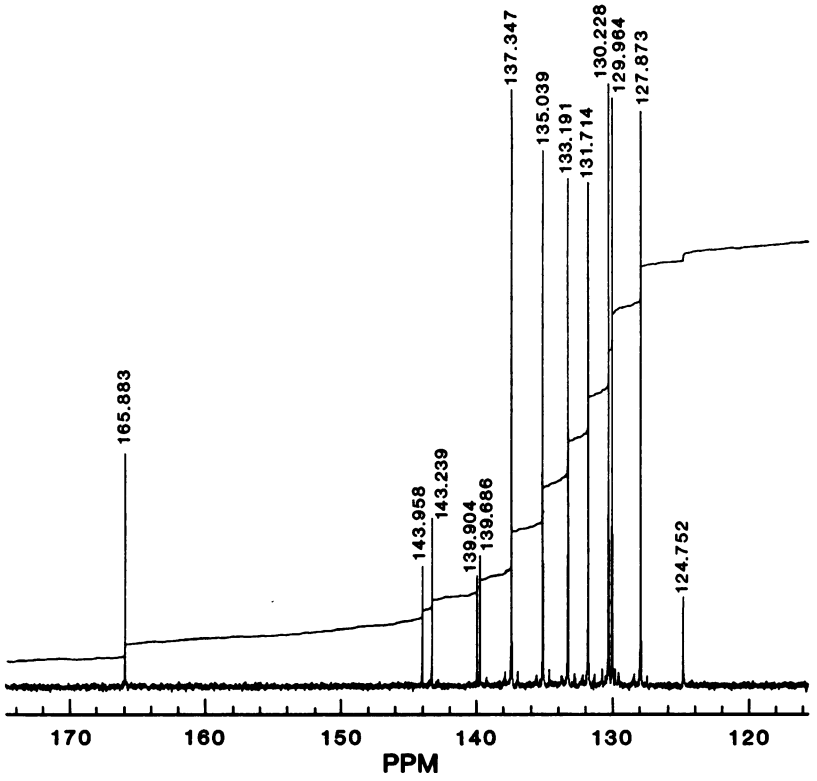


FIG. 4. ^{13}C-nuclear magnetic resonance of the antibiotic from *P. fluorescens* 2-79.

being profuse in some flasks and almost negligible or absent in others within the same batch of medium prepared under identical conditions and grown at the same time on the same shaker. An unknown factor probably is necessary to trigger the production of the yellow compound in the medium; the presence of excess iron in the medium may be one important factor. The variability in production of other phenazine compounds by *Pseudomonas* spp. has been reported previously (6).

Physical and chemical properties of the antibiotic. The antibiotic as purified was homogeneous by thin-layer chromatography under several solvent systems (Table 1). It was also homogeneous on the gas-liquid chromatographic column with a retention time of 1.20 min. The antibiotic was a greenish-yellow needle-shaped crystalline solid with a melting point of 242°C (decomposition). It was soluble in methylene chloride, chloroform, acetone, 2 N sodium hydroxide, and 2 N hydrochloric acid. It was insoluble in water, methanol, ethyl acetate, tetrahydrofuran, diethyl ether, carbon tetrachloride, hexane, and petroleum ether. The mass spectrum (Fig. 1). showed a molecular ion peak at *m/z* 448, which is consistent with a molecular formula of $C_{26}H_{16}N_4O_4$. Found: C, 69.26%; H, 3.62%; N, 12.63%; O, 14.15%. Calculated: C, 69.63%; H, 3.60%; N, 12.50%; O, 14.27%. The neutron activation analysis of the antibiotic showed the absence of Fe, Au, Cr, Co, and other heavy metal elements in the molecule and that the antibiotic molecule was mainly composed of the elements C, H, N, and O.

TABLE 2. ^{13}C chemical shifts of the antibiotic from *P. fluorescens* 2-79

Carbon[a]	Chemical shift
1,1′	124 · 752
2,2′	130 · 228
3,3′	137 · 347
4,4′	135 · 039
4a,4a′	139 · 686
5a,5a′	139 · 904
6,6′	131 · 714
7,7′	127 · 873
8,8′	129 · 964
9,9′	133 · 191
9a,9a′	143 · 239
10a,10a′	143 · 958
COOH	165 · 883

[a] The numbering system is indicated in Fig. 6, structure II.

The infrared spectrum (Fig. 2) of the compound showed the following major absorption bands: 3040, 3010 (aromatic H), 2900 to 2500 (broad), 1740 (ionized carboxylic acid or zwitter ion), 1625, 1605, 1565 and 1525 (C=C) cm^{-1}.

The mass spectrum of the compound (Fig. 1) showed that the initial loss of a molecule of carbon dioxide from a molecule of the antibiotic resulted in a fragment ion of *m/z* 404, and the loss of another carbon dioxide molecule resulted in a fragment of *m/z* 360 (Fig. 3). A base peak at *m/z* 180, which is one-half of *m/z* 360, coupled with the existence of a fragment ion at *m/z* 153 (which results from a loss of a HCN unit from *m/z* 180) indicates the presence of a phenazine moiety in the molecule. A fragment at *m/z* 360 strongly suggests the existence of a dimer. The existence of a phenazine moiety in the antibiotic molecule is further supported by a strong absorption peak at 251 nm accompanied by a broad peak at 364 nm (with shoulders at 353 and 409 nm), and also by the infrared bands at 860 and 840 cm^{-1} (2).

The mass spectrum of the compound also suggests the presence of two carboxylic acid groups in the molecule.

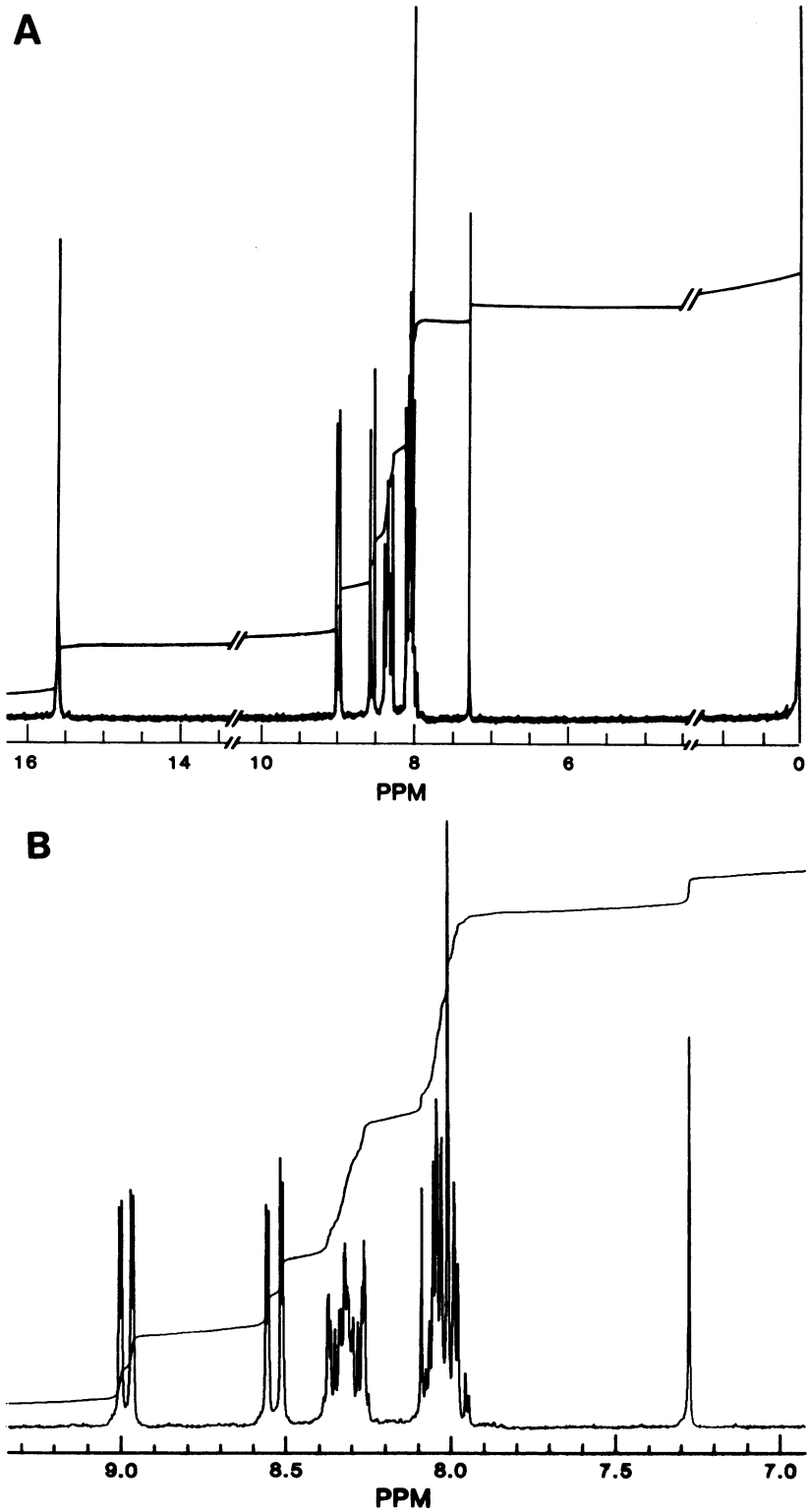


FIG. 5. (A) Proton magnetic resonance spectrum of the antibiotic from *P. fluorescens* 2-79. (B) Expanded proton magnetic resonance spectrum (7 to 9.5) of the antibiotic from *P. fluorescens* 2-79.

Loss of a hydroxyl radical from the molecular ion would give a fragment of *m/z* 431. Further loss of phenazine carboxylic acid (*m/z* 224) would give *m/z* 207. A loss of radical ion with *m/z* 223 from the parent molecular ion gives an even electron ion at *m/z* 225. Also, the presence of fragments at *m/z* 431 and *m/z* 207 would strongly support the presence of two carboxylic acid groups in the molecule (Fig. 1). This observation was further supported by proton magnetic resonance

and ^{13}C-nuclear magnetic resonance spectra of the compound.

The ^{13}C-nuclear magnetic resonance (proton noise decoupled) spectrum of the compound (Fig. 4) showed 13 well-resolved signals, indicating a very symmetrical structure for the molecule. The specific assignments were tentatively made in comparison with the reported data for substituted phenazines (1) (Table 2 and Fig. 4) and also on the basis of the proton magnetic resonance spectrum of the compound.

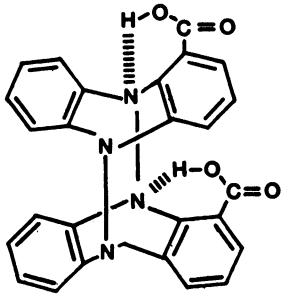
The proton nuclear magnetic resonance spectrum of the compound (Fig. 5A and B) exhibited an ABCD pattern in the aromatic ring, which is typical of phenazine derivatives (8). Comparison with previously reported values for 1-carbomethoxyphenazine (8) suggested that the most downfield resonance δ 8.95 to 9.00 belongs to the proton at position 4 (Table 3) of the phenazine ring. The next peak at δ 8.504 to 8.548 can be analogously assigned to the proton at position 2. These resonances appear as a doublet of doublets (δ 8.99 [J = 8.7 Hz, 1.46 Hz] and δ 8.526 [J = 7 Hz, 1.46 Hz]). As shown by the decoupling experiments, irradiation at one of these resonance positions removed the smaller coupling (J = 1.46 Hz) from the other. This established their *meta*-coupling. The proton at position 3 resonated in the same region as those at positions 6 and 9 (δ 8.310 to 8.380). The remaining two protons at positions 7 and 8 of the phenazine ring resonated at δ 7.939 to 8.302. The low-field proton singlet at δ 15.579 was readily exchangeable with D_2O and represented the hydrogen-bonded carboxylic acid protons.

The arrangement of protons in the molecule as described above, coupled with the other spectral properties, led us to conclude that the two phenazine moieties in the antibiotic molecule are very likely linked through nitrogen atoms. The spectral data can be explained in terms of three possible structures (I, II, and III; Fig. 6). All three structures had significant symmetry elements such as (i) mirror plane (I), (ii) center of inversion (II), and (iii) C_2 axis (III), which are required from the symmetrical ^1H- and ^{13}C-nuclear magnetic resonance spectra. Of the three possible isomers, structure

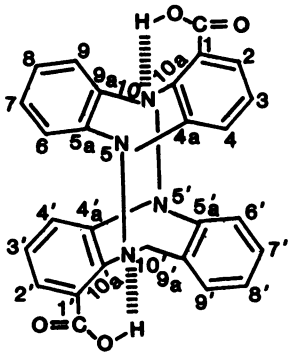
TABLE 3. ^1H-nuclear magnetic resonance spectra of the antibiotic from *P. fluorescens* 2-79[a]

Assignment[b]	Protons	Irradiation	
		δ 8.98	δ 8.53
COOH	15.579 s, 2H (D_2O exchangeable)	s	s
C_4—H	8.98, dd, 2H (J = 8.7, 1.46 Hz)		d (J = 7 Hz)
C'_4—H			
C_2—H	8.53, dd, 2H (J = 7, 1.46 Hz)	d (J = 8.7 Hz)	
C'_2—H			
C_3-H			
C'_3—H	8.310–8.380, m, 4H	m	m
$C_{7,8}$—H			
$C'_{7,8}$—H			
C_6—H			
C'_6—H	7.939–8.302, m, 6H	m	m
C_9—H			
C'_9—H			

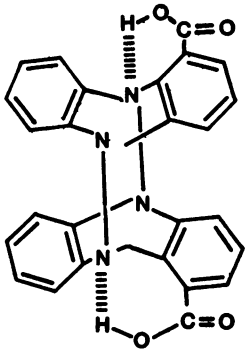
[a] Abbreviations: s, singlet; dd, doublet of doublet; d, doublet; m, multiplet.
[b] The numbering system is indicated in Fig. 6, structure II.



I



II



III

FIG. 6. Probable structures of the antibiotic from *P. fluorescens* 2-79: I (mirror plane), II (center of inversion), and III (C_2-axis).

II (Fig. 6) seems to be most fitting because of its higher symmetry.

Because all the protons on the phenazine moiety could be assigned, it is most likely that the two phenazine moieties must be joined by N—N bonds to form a dimeric structure identified by the molecular ion peak at m/z 448 of the mass spectrum (Fig. 1). Such dimers could form by a thermally allowed eight-electron cyclo addition reaction analogous to that of the anthracene dimer, and similar dimers are also known to form in phenazines (3). Furthermore, the lithium aluminum hydride reduction of the antibiotic yielded 1-hydroxymethyl phenazine as a major product, which is consistent with the proposed structures (I, II, and III; Fig. 6).

The structural features and spectral properties of the antibiotic isolated from *P. fluorescens* are similar to those of the antibiotic tubermycin B (phenazine-1-carboxylic acid) (2). However, the solubility properties, molecular weights,

infrared absorption bands, especially at fingerprinting regions, and the biological properties of these two compounds are significantly different.

Biological properties of the antibiotic. The antibiotic showed excellent activity against several fungi, including wheat pathogens such as *G. graminis* var. *tritici*, *Rhizoctonia solani*, and *Pythium aristosporum* (Table 4). It also exhibited moderate activity on several genera of bacteria (Table 5). It may have a practical value in the control of seedling diseases caused by *Pythium* species and take-all caused by *G. graminis* var. *tritici*, both of which are of considerable economic importance. The lithium aluminum hydride reduction of the antibotic resulted in the formation of three or four products of the antibiotic, of which one derivative, the hydroxymethyl phenazine, retained most of the biological characteristics of the parent molecule. Because this derivative was more soluble than the parent compound, it may be more ideal for use in disease control.

Recently, Weller et al. (Phytopathology **75**:1301, 1985) reported that mutants of strain 2-79 that lost in vitro inhibition of *G. graminis* var. *tritici* also lost in vivo suppressiveness of take-all. We suggest that production of the compound described herein may be one mechanism by which strain 2-79 is able to suppress take-all of wheat. Strain 2-79 appears to produce other phenazines under different conditions, and they may also have a role in suppression of take-all (P. G. Brisbane and A. D. Rovira, Abstr. Meet. Australas. Plant Pathol. Soc. 1985). The ability of *Pseudomonas* spp. to produce more than one phenazine is known (6, 10).

The mice that received the parent antibiotic compound by oral doses up to 464 mg/kg showed no symptoms because of toxicity. However, absence of toxicity at these concentrations may not mean that the compound is nontoxic to mice, because it may not be absorbed through the intestinal system. To confirm the absence of toxicity, the antibiotic should be administered to mice intraperitoneally or intravenously.

TABLE 4. Antifungal spectrum of the compound from *P. fluorescens* 2–79

Test fungus	MICs (μg/ml)[a]
Cochliobolus sativus	1–3
Coniophora puteana	30–40
Corticium galactinum	7–10
Cytospora sp.	15–20
Cytospora decipiens	30–40
Fomes officinalis	7–10
Fomes pini	30–40
Fusarium sp.	25–30
Gaeumannomyces graminis var. *tritici*	1
Hydnum abietis	15–20
Pholiota adiposa	7–10
Pholiota alnicola	7–10
Polyporus abietinus	7–10
Polyporus picipes	7–10
Polyporus schweinitzii	7–10
Poria placenta	7–10
Poria subincarnata	3–5
Poria weirii	10–15
Poria xantha	7–10
Pythium aristosporum	1
Pythium species E (heterothallic) (male)	3–5
Pythium species E (heterothallic) (female)	3–5
Pythium heterothallicum (male)	1
Pythium heterothallicum (female)	3–5
Pythium irregulare	10–15
Pythium sylvaticum	10–15
Pythium torulosum	10–15
Pythium ultimum	25–30
Pythium ultimum var. *sporangiiferum*	80–100
Pythium volutum	1
Rhizoctonia solani	1
Rigidoporus nigrescens	3–5
Schizophyllium commune	15–20
Stereum chailletii	7–10
Stereum sanguinolentum	30–40
Stereum sulcatum	7–10
Stereum sp.	15–20
Trametes serialis	3–5
Trametes variiformis	3–5
Trechispora raduloides	30–40
Trechispora sp.	30–40
Vararia granulosa	7

[a] Total inhibition.

TABLE 5. Antibacterial spectrum of the antibiotic isolated from *P. fluorescens* 2–79

Organism	Growth-inhibitory concentration of the antibiotic (μg/ml)
Actinomyces viscosus	100
Bacillus subtilis	<50
Bacteroides fragilis	50
Bacteroides multiacidus	>100
Clostridium perfringens	>100
Clostridium septicum	100
Erwinia amylovora	>100
Escherichia coli	>100
Fusobacterium necrophorum	>1.0
Lactobacillus acidophilus	>100
Micrococcus lutea	>100
Pseudomonas aeruginosa	>100
Salmonella typhimurium	>100
Staphylococcus aureus	>100
Streptococcus faecalis	>100
Streptococcus mutans	<25
Streptococcus bovis	<50

LITERATURE CITED

1. **Breitmaier, E., and U. Hollstein.** 1976. Carbon-13 nuclear magnetic resonance chemical shifts of substituted phenazines. J. Org. Chem. **41**:2104–2108.
2. **Chang, P. C., and A. C. Blackwood.** 1969. Simultaneous production of three phenazine pigments by *Pseudomonas aeruginosa* Mac 436. Can. J. Microbiol. **15**:439–444.
3. **Eremenko, S. M., I. A. Dolidze, and B. Y. Dain.** 1973. Parallel study of photochemistry and luminescence of phenazine. Khim. Vys. Energ. **7**:276–278.
4. **Gurusiddaiah, S., L. D. Winward, D. Burger, and S. O. Graham.** 1979. Pantomycin: a new antimicrobial antibiotic. Mycologia **71**:103–118.
5. **Houghland, G. V. C., and L. C. Cash.** 1954. The use of vermiculite in providing scab inoculation for potatoes. Plant Dis. Rep. **38**:460–461.
6. **Kanner, D., N. N. Gerber, and R. Bartha.** 1978. Pattern of phenazine pigment production by a strain of *Pseudomonas aeruginosa*. J. Bacteriol. **134**:690–692.
7. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301–307.
8. **Römer, A.** 1982. ^{1}H NMR spectra of substituted phenazines. Org. Magnetic Resonance **19**:66–68.
9. **Shipton, P. J.** 1975. Take-all decline during cereal monoculture,

p. 137–144. *In* G. W. Bruehl (ed.), Biology and control of soil-borne plant pathogens. American Phytopathological Society, St. Paul, Minn.

10. **Toohey, J. I., C. D. Nelson, and G. Krotkov.** 1965. Isolation and identification of two phenazines from a strain of *Pseudomonas aureofaciens*. Can. J. Bot. **43**:1055–1062.
11. **Vidaver, A. K.** 1967. Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: effect of carbon source. Appl. Microbiol. **15**:1523–1524.
12. **Weller, D. M., and R. J. Cook.** 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology **73**:463–469.