A New Chlorinated Phenylpyrrole Antibiotic Produced by the Antifungal Bacterium *Pseudomonas cepacia*

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A group of chlorinated phenylpyrrole derivatives was isolated from a strain of *Pseudomonas cepacia* collected from apple leaves during a screening program designed to detect agents for biological control of fruit spoilage fungi. One of these substances, 2,3-dichloro-4-(2-amino-3-chlorophenyl)pyrrole, has not been previously reported. In vitro testing showed that all four of the phenylpyrroles had antifungal activity toward several fruit pathogens. The new phenylpyrrole showed fungal inhibitory effects on Golden Delicious apples inoculated with conidia of pathogenic organisms. An unrelated but known compound, 2-(2-heptenyl)-3-methyl-4(1*H*)-quinolone, was also isolated.

Recently there has been a resurgence of interest in finding and developing biological control methods as alternatives to fungicidal treatments currently employed to control fruit pathogens (Janisiewicz, 1987; Wilson and Pusey, 1985). In part, this renewed attention has resulted from increasing pathogen resistance to fungicides (Spotts and Cervantes, 1986) and growing concern over potential health hazards associated with pesticide residues in the human food chain (Board of Agriculture, 1987). As part of a program designed to develop biological control procedures for some fruit pathogens, a bacterium (later identified as *Pseudomonas cepacia* Burkh) isolated from apple leaves was shown to be antagonistic toward several prominent fruit spoilage pathogens (Janisiewicz and Roitman, 1987, 1988). We have grown this strain of *P. cepacia* (LT4-12-W) in shake cultures and now describe the isolation and characterization of five metabolites: one quinolone and four chlorine-containing phenylpyrroles, one of which has not previously been reported.

EXPERIMENTAL SECTION

Materials and Equipment. NMR spectra were obtained in $CDCl_3$ on a Nicolet NT-WB200 FT spectrometer at 200 MHz (^1H) and at 50 MHz (^{13}C). Carbon spectra were obtained with a pulse sequence (Patt and Schoolery, 1982) supplied with the instrument software so that quaternary and methylene carbons were distinguished from methyl and methine carbons. Mass spectra were obtained at 70 eV on a VG Micromass 7070 spectrometer at a source temperature of 120 °C. A Hewlett-Packard 8451A diode array spectrophotometer was used for UV measurements. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected.

Growth medium was prepared from reagent-grade chemicals and distilled deionized water. Amberlite XAD-4 and XAD-7 macroreticular resins (Rohm and Haas) were washed with water and extracted in a Soxhlet apparatus with acetone prior to use. HPLC-grade solvents were used throughout both workup and purification procedures. Reversed-phase C_{18} glass TLC plates (Whatman) were developed in $CH_3OH/CH_3CN/H_2O$ (1:1:1) and visualized by short-wavelength UV light and by spraying with diazotized sulfanilic acid (DSA). HPLC experiments were performed on an IBM LC/9533 instrument.

Incubation in Shake Culture. A minimal broth was prepared by dissolving the following quantities of ingredients (grams per liter) in water: NH_4Cl, 3; KH_2PO_4, 21.8; Na_2HPO_4, 5.7; $MgSO_4 \cdot 7H_2O$, 0.5; $ZnSO_4 \cdot 7H_2O$, 0.05; $FeSO_4 \cdot 7H_2O$, 0.5; glycerol, 31; monosodium glutamate, 10. Fernbach flasks (2800 mL) were charged with 500 mL of the above mixture covered, with two gauze milk filters topped by a large square of brown paper secured with rubber bands and sterilized at 121 °C for 15 min. After being cooled to ambient temperature, the flasks were inoculated from agar cultures of LT4-12-W strain of *P. cepacia* and shaken at 250 rpm on a gyro shaker at 27 °C for 7 days.

Isolation and Identification of Metabolites. The fermentation broth was centrifuged at 10000*g* for 15 min and the supernatant liquid decanted and discarded. The precipitated cells were suspended in acetone, sonicated at high power (Branson sonifier 450) for 1 min, and recentrifuged, and the precipitated material was discarded. The aqueous acetone solution was then stripped of acetone by rotary evaporation at <50 °C and the remaining aqueous solution freeze-dried to give an amorphous brown solid.

The crude product mixture was then dissolved in methanol, filtered, concentrated, and refiltered through a 0.45-μm Durapore filter (Millipore) before injection onto a C_{18} reversed-phase HPLC column: Rainin Dynamax, 8 μm, 21.4 × 250 mm with 50-mm guard column; flow, 20 mL/min; solvent, CH_3CN/H_2O (3:2, v/v); UV detector, 254 nm.

Five partially purified fractions corresponding to chromatographic peaks (UV) were isolated from the crude mixture of metabolites by preparative HPLC. The five fractions were collected at the following time intervals after injection: (1) 6.5–7.5 min; (2) 7.5–8.5 min; (3) 9.5–12.5 min; (4) 12.5–13.5 min; (5) 13.5–15.5 min. Fractions 1 and 2 were further purified on a cyanosilica HPLC column (IBM 9 × 250 mm) with $CHCl_3$/hexane (1:1) at a flow rate of 5 mL/min (crude fraction 2) and $CHCl_3$/MeOH (97:3) at 3 mL/min (crude fraction 1). Fractions 3 and 5 were purified on a silica HPLC column (Rainin Dynamax, 8 μm, 21.4 × 250 mm) with $CHCl_3$/hexane (1:1) at 20 mL/min to elute pure substances from fraction 3 at 13.5 min and from fraction 5 at 11.0 min. Purification of fraction 4 on the same column with $CHCl_3$/hexane/MeOH (14:6:1) at 20 mL/min afforded a single substance at 6.2 min.

2,3-Dichloro-4-(2-amino-3-chlorophenyl)pyrrole (1). Evaporation of the solvent from the rechromatographed principal peak from crude fraction 4 above, followed by recrystallization of the residue from $CHCl_3$/hexane, afforded colorless crystals: mp 119–120 °C; R_f 0.28, orange spot with DSA; mass spectrum, *m/z* (rel intens) 259.9672 (M^+, 99) [$C_{10}H_7{}^{35}Cl_3N_2$ requires 259.9675], 266 (3), 264 (32), 263 (13), 262 (100), 261 (13), 232 (8), 228 (16), 227 (47), 226 (68), 225 (74), 224 (90), 200 (28), 199 (15), 198 (45), 197 (18), 192 (18), 191 (33), 190 (56), 189 (86), 164 (13), 163 (17), 162 (30), 161 (10), 155 (15), 153 (12), 128 (10), 127 (19), 126 (25), 100 (14), 99 (19), 95 (11), 76 (12), 75 (16), 74 (13), 63 (14), 52 (17), 51 (12), 50 (10); ^1H NMR, δ 4.22 (br s, 2 H, NH_2), 6.71 (t, *J* = 8 Hz, 1 H, H5′), 6.76 (d, *J* = 3 Hz,

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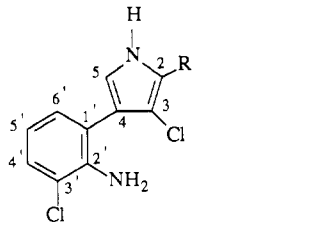

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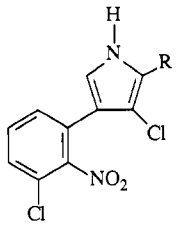

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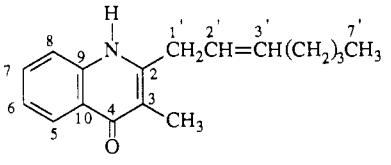

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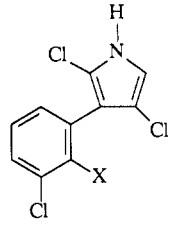

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1 H, H5), 7.05 (dd, J = 7.7, 1.7 Hz, 1 H, H6′), 7.25 (dd, J = 7.7, 1.7 Hz, 1 H, H4′), 8.35 (br s, 1 H, NH); ^{13}C NMR, δ 141.6 (C2′), 129.8 (C4′), 128.8 (C6′), 120.1 (C3′), 119.53 (C1′ or C4), 119.47 (C4 or C1′), 118.0 (C5′), 115.4 (C5), 112.8 (C2 or C3), 109.2 (C3 or C2); UV [MeOH; λ_{max}, nm (log ε)] 212 (4.54), 302 (3.61).

2,3-Dichloro-4-(2-nitro-3-chlorophenyl)pyrrole (2). The collected rechromatographed major substance from fraction 5 gave, after recrystallization from $CHCl_3$/hexane, pale yellow crystals: mp 142–143 °C (lit. mp 143–144 °C (Hamill et al., 1967)]; R_f 0.22, orange spot with DSA; mass spectrum, m/z (rel intens) 289.9414 (M^+, 29) [$C_{10}H_5{}^{35}Cl_3N_2O_2$ requires 289.9416], 296 (1), 294 (10), 292 (28), 264 (11), 262 (11), 229 (19), 228 (11), 227 (16), 210 (15), 209 (11), 208 (20), 204 (13), 203 (14), 202 (63), 201 (24), 200 (100), 199 (13), 193 (8), 191 (12), 184 (14), 182 (11), 176 (10), 175 (11), 174 (28), 173 (19), 172 (33), 171 (12), 166 (28), 165 (11), 164 (55), 163 (18), 162 (12), 161 (12), 149 (12), 147 (22), 139 (11), 138 (24), 137 (21), 136 (21), 110 (15), 105 (11), 99 (10), 87 (12), 86 (17), 73 (11), 62 (12); ^{1}H NMR, δ 6.75 (d, J = 3 Hz, 1 H, H5), 7.48 (s, 3 H, H4′, H5′, H6′), 8.41 (br s, NH); ^{13}C NMR, δ 149.0 (C2′), 130.3 (C4′ or C5′), 130.1 (C5′ or C4′), 129.1 (C6′), 127.0 (C1′), 124.9 (C3′), 116.1 (C4), 115.7 (C5), 113.6 (C2 or C3), 109.0 (C3 or C2); UV [MeOH; λ_{max}, nm (log ε)] 212 (4.47), 240 (sh), 280 (sh).

Reduction of 2. A solution of 2,3-dichloro-4-(2-nitro-3-chlorophenyl)pyrrole (100 mg) in EtOAc (10 mL) containing HOAc (0.1 mL) was stirred with 5% Pd on C (25 mg) under H_2 (1 atm) at ambient temperature for 36 h. After removal of the catalyst by filtration and solvent by rotary evaporation, the remaining residue was purified by HPLC [silica, 21.4 × 250 mm, $CHCl_3$/hexane/MeOH (14:6:1)] and recrystallization to give colorless crystals (76 mg), mp 119–120 °C, chromatographically and spectroscopically identical with 2,3-dichloro-4-(2-amino-3-chlorophenyl)pyrrole (1).

3-Chloro-4-(2-amino-3-chlorophenyl)pyrrole (3). Rechromatography of crude fraction 2, collection of the major substance, and recrystallization from $CHCl_3$/hexane afforded colorless crystals: mp 91–92 °C [lit. mp 88–90 °C (Hamill et al., 1967)]; R_f 0.38, maroon spot with DSA; mass spectrum, m/z (rel intens) 226.0057 (M^+, 100) [$C_{10}H_8{}^{35}Cl_2N_2$ requires 226.0064], 230 (11), 228 (65), 198 (7), 193 (21), 192 (29), 191 (67), 190 (71), 164 (24), 163 (18), 156 (74), 155 (28), 129 (10), 128 (25), 127 (14), 102 (11), 101 (17), 96 (15), 95 (21), 78 (20), 77 (19), 75 (12), 64 (10); ^{1}H NMR, δ 4.24 (br s, 2 H, NH_2), 6.72 (t, J = 7.8 Hz, H5′), 6.80 (t, J = 2.5 Hz, H2), 6.85 (t, J = 2.8 Hz, H5), 7.09 (dd, J = 1.5, 7.6 Hz, H6′), 7.24 (dd, J = 1.5, 7.6 Hz, H4′), 8.35 (br s, 1 H, NH). ^{13}C NMR, essentially identical with that reported in the literature (Martin et al., 1972); UV [MeOH; λ_{max}, nm (log ε)] 212 (4.46), 302 (3.57).

3-Chloro-4-(2-nitro-3-chlorophenyl)pyrrole (Pyrrolnitrin, 4). After rechromatography of fraction 3 and recrystallization from $CHCl_3$/hexane, pyrrolnitrin was collected as fine yellow crystals: mp 124–125 °C [lit. mp 124.5 °C (Arima et al., 1964)]; R_f 0.31, maroon spot with DSA; mass spectrum, m/z (rel intens) 255.9806 (M^+, 100) [$C_{10}H_6{}^{35}Cl_2N_2O_2$ requires 255.9806], 260 (11), 258 (61), 231 (23), 230 (15), 229 (38), 228 (21), 205 (16), 203 (23), 202 (15), 201 (35), 200 (21), 195 (24), 194 (13), 193 (71), 185 (12), 184 (15), 183 (14), 177 (24), 176 (15), 175 (52), 174 (29), 172 (31), 168 (24), 167 (20), 166 (80), 165 (49), 164 (26), 163 (13), 162 (11), 150 (18), 149 (18), 148 (36), 147 (23), 140 (64), 139 (32), 138 (70), 137 (70), 136 (45), 135 (18), 130 (11), 129 (11), 128 (11), 127 (11), 114 (20), 113 (59), 112 (13), 111 (18), 110 (31), 102 (26), 101 (12), 100 (12), 99 (24), 97 (12), 88 (18), 87 (52), 86 (22), 75 (29), 74 (35), 73 (18), 69 (16), 64 (21), 63 (31), 62 (14), 55 (10); ^{1}H NMR, δ 6.81 (m, 2 H, H2, H5), 7.41 (m, 1 H, H6′), 7.43 (m, 1 H, H5′), 7.52 (m, 1 H, H4′), 8.38 (br s, 1 H, NH) [the complex 11-line pattern of H4′, H5′, and H6′ was reproduced by computer simulation using J_{ortho} = 8 Hz and J_{meta} = 2 Hz]; ^{13}C NMR, identical with that reported (Martin et al., 1972) for pyrrolnitrin; UV [MeOH; λ_{max}, nm (log ε)] 212 (4.39), 252 (3.83).

2-(2-Heptenyl)-3-methyl-4(1*H*)-quinolone (5). Rechromatography of crude fraction 1 and recrystallization from MeOH/H_2O afforded colorless crystals: mp 243–244 °C (lit. mp 255 °C dec (Hashimoto and Hattori, 1967)]; R_f 0.35, colorless with DSA; ^{13}C NMR, δ 10.6 (C3-Me), 13.9 (C7′), 22.2 (C6′), 31.3 (C5′), 32.2 (C4′), 35.6 (C1′), 115.6 (C3), 117.3 (C8), 123.0 (C6), 123.3 (C3′), 123.6 (C10), 125.8 (C5), 131.1 (C7), 135.5 (C2′), 139.2 (C9), 147.9 (C2), 178.0 (C4); UV, agrees well with literature values (Hashimoto and Hattori, 1967).

RESULTS AND DISCUSSION

Isolation and Identification of the Major Antifungal Metabolite Pyrrolnitrin (4). The first experiments performed with culture LT 4-12-W grown on nutrient broth–yeast extract medium in shake culture showed that although cell-free culture filtrates showed no antifungal activity (agar diffusion test; Janisiewicz and Roitman, 1988), a highly active fraction could be separated from the total filtrate by adsorption on a macrorecticular polymer resin (Amberlite XAD-4 or XAD-7) followed by desorption with methanol. Bioassay of chromatography fractions permitted correlation of antifungal activity (against *Penicillium expansum*, *Botrytis cinerea*, and *Mucor periformis*) with one reversed-phase HPLC fraction comprised of a single substance. Initially, very small quantities of this substance were obtained (<1 mg/L of culture filtrate); however, the mass spectrum provided the elemental composition $C_{10}H_6{}^{35}Cl_2N_2O_2$ and suggested pyrrolnitrin (4) as a likely structure. Repeated culturing and isolation provided sufficient material for NMR spectra, which along with melting point and UV spectral data confirmed the identity as pyrrolinitrin (4). Subsequently, we found that use of a minimal salts medium produced much more pyrrolnitrin. Furthermore, most of it was found in the cells, permitting the voluminous broth to be discarded after separation of the cells by centrifugation.

During repeated preparation of pyrrolnitrin for antifungal testing on fruit, several other minor peaks were observed in the HPLC chromotograms. These were collected and repurified to homogeneity, and the substances responsible for these HPLC peaks were identified as follows.

Lesser Phenylpyrrole Metabolites 1–3. When thin-layer chromatograms of these metabolites were sprayed with diazotized sulfanilic acid, orange spots were produced by 1 and 3 and purple spots by 2 and 4. The amino analogue 3 of pyrrolnitrin had been previously isolated from *Pseudomonas aureofaciens* and given the trivial name amino pyrrolinitrin (Hamill et al., 1967). It had also been prepared from pyrrolnitrin by catalytic reduction (Martin et al., 1972). Comparison of reported physical con-

stants and spectra with our own confirmed the identity of 3.

Mass spectra of the remaining two metabolites displayed isotopic clusters of molecular ions strongly indicative of molecules having three chlorine atoms (McLafferty, 1967). This supposition was confirmed by high-resolution mass measurements, which provided elemental compositions $C_{10}H_7{}^{35}Cl_3N_2$ for 1 and $C_{10}H_5{}^{35}Cl_3N_2O_2$ for 2. ^1H NMR spectra of 1 and 2 showed signals for only four aromatic protons mirrored by the presence of but four CH signals in the ^{13}C spectrum. Comparison of the NMR spectra of 3 and 4 with those of 1 and 2 and the molecular formulas indicated that replacement of an aromatic proton from 3 or 4 with a chlorine atom would produce, respectively, 1 or 2. The fact that the ^1H NMR spectra of each of the four substances shows three contiguous aromatic CH signals corresponding to H4′, H5′, and H6′ demonstrates that the third Cl of 1 and 2 is on the pyrrole ring.

Although 2 has been reported from *P. aureofaciens*, no physical data other than the melting point and molecular weight were given (Hamill et al., 1967); the authors assigned the additional chlorine to the pyrrole ring because only a single pyrrole proton signal was observed in the ^1H NMR spectrum (spectrum not reported). Because the alternative structure 6 cannot be ruled out from the published evidence, some proton–proton decoupling experiments were performed on the spectrum of 1 to determine whether long-range coupling between H6′ and H5 could be detected. In fact, when H6′ (δ 7.05) was irradiated, the pyrrole proton doublet (δ 6.76) noticeably sharpened, confirming that weak, long-range coupling between the two protons exists. These data indicate that five bonds, as in 1 and 2, rather than six, as in 6, intervene between H6′ and H5.

The structure of 1 was further supported by the pattern of ions displayed in its mass spectrum. All four phenylpyrrole compounds have mass spectral ions corresponding to the expulsion from the pyrrole ring of a neutral fragment comprised of the heterocyclic nitrogen and either of the two adjacent carbons (Figure 1). All of the substances eject HN–CH whereas 1 and 2 eliminate HN–CCl as well. The resulting ions, which can formally be considered to be cyclopropenium ions, undergo further decomposition by loss of H• or Cl• as shown.

The relationship between 1 and 2 was further established by catalytic reduction (Pd on C) of 2 to produce 1 in high yield. This material was identical with that isolated from *P. cepacia* cells. As expected, the corresponding benzene ring ^{13}C signals for amino derivatives 1 and 3 are identical and the benzene carbon signals of nitro derivatives 2 and 4 are also identical with one another.

It is surprising that 1 has never been reported previously, since the biosynthesis of pyrrolnitrin (4) from tryptophan has been shown (Martin et al., 1972) to involve the intermediate amino compound 3.

We have isolated an additional substance, quinolone 5, which has been previously reported (Hashimoto and Hattori, 1967). Its ^{13}C NMR spectrum has not been published yet and is presented here (Experimental Section); the signals were assigned after comparison with spectra of model quinolones (Shamma and Hindenlang, 1979). In vitro testing against *P. expansum*, *B. cinerea*, and *Mucor* sp. by the agar diffusion method showed no antifungal activity for 5.

Preliminary biological evaluation of the effect of 1 on controlling spoilage of wounded Golden Delicious apples was accomplished by a method described earlier (Janisiewicz and Roitman, 1988). Fruit was wounded (holes of 3-mm diameter × 3-mm depth), and the wounds were treated with aqueous solutions of 1 (20 μL) at various concentrations (0–250 ppm). The wounds were then inoculated with conidial suspensions (20 μL × 10^4 mL^{-1}) of three prominent fruit pathogens, *P. expansum*, *B. cinerea*, or *Rhizopus* sp. The fruit were maintained at 24 °C for 7 days, and the diameter of the lesion radiating from the wound was measured visually. At concentrations of 1 below 50 ppm, treated and control fruit were identical, but at 200 ppm *Rhizopus* lesions were eliminated and *Penicillium* and *Botrytis* lesions were reduced in diameter by 80%; at 250 ppm all lesions were eliminated. Further testing is currently under way.

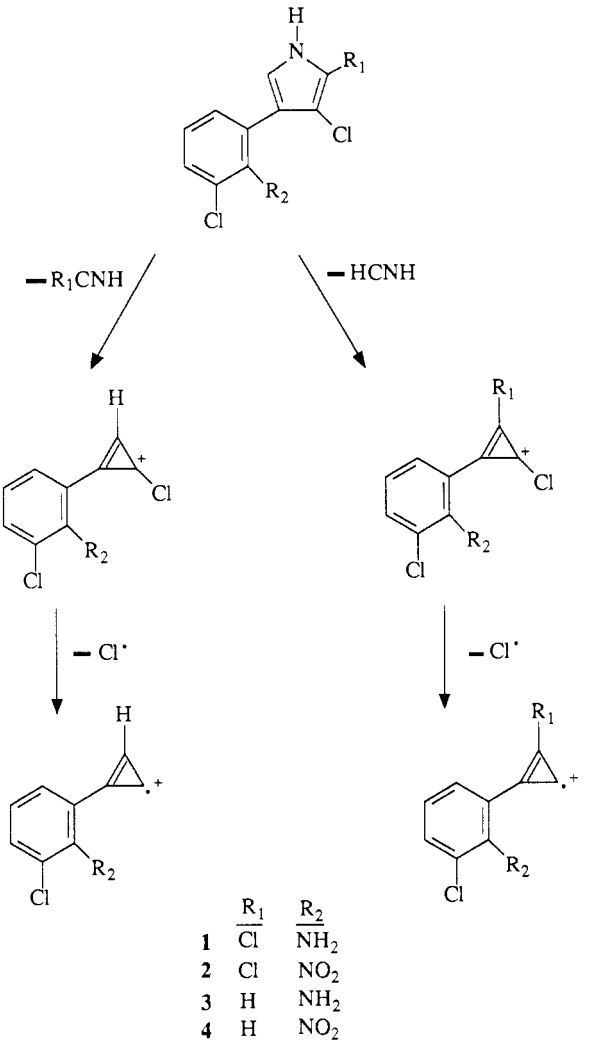

Figure 1. Mass spectral fragments of *P. cepacia* phenylpyrroles.

LITERATURE CITED

Arima, K.; Imanaka, H.; Kousaku, M.; Fukuta, A.; Tamura, G. Pyrrolnitrin, a New Antibiotic Substance, Produced by a *Pseudomonas*. *Agric. Biol. Chem.* **1964**, *28*, 575.

Board of Agriculture. *Regulation Pesticides in Food - The Delaney Paradox*; National Research Council, National Academy Press: Washington, DC, 1987; 272 pp.

Hamill, R.; Elander, R.; Mabe, J.; Gorman, M. Metabolism of Tryptophans by *Pseudomonas aureofaciens*. *Antimicrob. Agents Chemother.* **1967**, 388.

Hashimoto, M.; Hattori, K. 2-(2-Heptenyl)-3-methyl-4-quinolinol from a *Pseudomonas*. *Chem. Pharm. Bull.* **1967**, *15*, 718.

Janisiewicz, W. J. Biological Control of Diseases of Fruits. In *Biological Control of Plant Diseases*; Mukerji, R. J., Gary, K. L., Eds.; CRC Press: Boca Raton, FL, 1987; Vol. II, pp 153–165.

Janisiewicz, W. J.; Roitman, J. N. Postharvest *Mucor* Rot Control on Apples with *Pseudomonas cepacia*. *Phytopathology* **1987**, *77*, 1776.

Janisiewicz, W. J.; Roitman, J. N. Biological Control of Blue Mold and Gray Mold on Apple and Pear with *Pseudomonas cepacia*. *Phytopathology* **1988**, *78*, 1697.

Martin, I. I.; Chang, C.-J.; Floss, H. G.; Mabe, J. A.; Hagaman, E. W.; Wenkert, E. A ^{13}C Nuclear Magnetic Resonance Study on the Biosynthesis of Pyrrolnitrin from Tryptophan by *Pseudomonas*. *J. Am. Chem. Soc.* **1972**, *94*, 8942.

McLafferty, F. W. Molecular Formulas. *Interpretation of Mass Spectra*; Benjamin: New York, 1967; p 22.

Patt, S. L.; Schoolery, J. N. Attached Proton Test for Carbon-13 NMR. *J. Magn. Reson.* **1982**, *46*, 535.

Shamma, M.; Hindenlang, D. *Carbon-13 Shift Assignments of Amines and Alkaloids*; Plenum: New York, 1979; pp 87–89.

Spotts, R. A.; Cervantes, L. A. Populations, Pathogenicity, and Benomyl Resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in Packinghouses. *Plant Dis.* **1986**, *70*, 106–108.

Wilson, C. L.; Pusey, P. L. Potential for Biological Control of Postharvest Plant Diseases. *Plant Dis.* **1985**, *69*, 375–378.

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Synthesis and Herbicidal Activity of 1-Aryl-5-halo- and 1-Aryl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides

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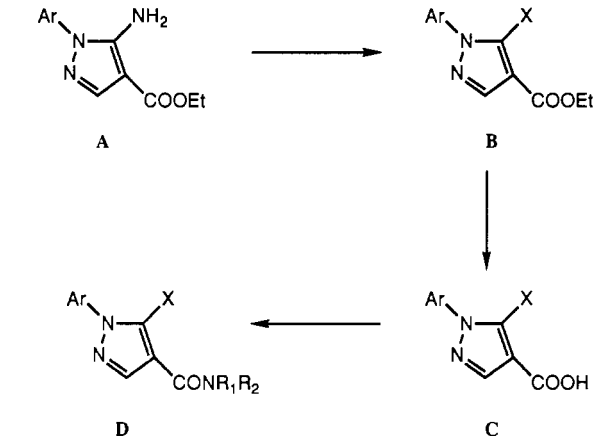
A series of 1-aryl-5-halo- and 1-aryl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides exhibit moderate to strong herbicidal activity in preemergence and postemergence tests. At $^1/_2$ lb/acre, corn, rice, wheat, cotton, and soybean show tolerance, while large crabgrass, foxtail millet, common lambsquarters, redroot pigweed, wild mustard, velvetleaf, jimsonweed, and zinnia were killed or severely injured. A total of 83 5-halo analogues and 47 5-trifluoromethyl analogues were synthesized and their herbicidal activities determined in order to examine the structure–activity relationships. The order of activity at C-5 of the pyrazole ring was CF_3 > Cl ≅ Br > I. The order of activity involving substitution on the carboxamide moiety was cyclopropyl ≅ methyl > dimethyl > ethyl > isopropyl. Substitution on the benzene ring did not result in any major increase in activity when compared with the corresponding phenyl analogue.

1-Aryl-5-halo-1*H*-pyrazole-4-carboxamides and the corresponding 5-trifluoromethyl analogues represent a new class of preemergence and postemergence herbicides (Beck and Lynch, 1986). The 5-halo derivatives were synthesized in three steps (Scheme I) from ethyl 5-amino-1-aryl-1*H*-pyrazole-4-carboxylate esters (**A**) which were prepared by the reaction of ethyl (ethoxymethylene)cyanoacetate with the appropriate arylhydrazine (Beck et al., 1987 and references therein). The conversion of the amino esters to the halo esters (**B**) by a process involving nonaqueous diazotization was reported in a U.S. Patent (Beck and Lynch, 1986) and a recent publication (Beck et al., 1987). Treatment of the amino esters with nitrosyl chloride in chloroform gave the corresponding chloro esters (**B**, X = Cl). Similar treatment with isopentyl nitrite in the presence of bromine or iodine led to the formation of the bromo and iodo esters (**B**, X = Br and I), respectively. The halo esters were converted to the carboxylic acids (**C**) by base hydrolysis, and the herbicidal halo carboxamides (**D**) were prepared by standard procedures.

The 5-trifluoromethyl analogues were synthesized in three steps (Scheme II) from ethyl 3-ethoxy-2-(trifluoroacetyl)-2-propenate (**A**) (Jones, 1951). Treatment of **A** with the appropriate arylhydrazine under mild conditions led to the formation of pyrazole-4-carboxylate esters **B** (Beck and Lynch, 1986; Beck and Wright, 1987). Saponification gave carboxylic acids **C**, which were converted

Scheme I



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