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Chemistry

Phytotoxins from the pathogenic fungi *Drechslera maydis* and *Drechslera sorghicola*

(corn blight/fungal metabolites/plant disease/sesterterpenoids/ophiobolins)

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ABSTRACT **_Drechslera maydis_, the causal agent of Southern corn leaf blight, and _Drechslera sorghicola_, the causal agent of leaf spot on Johnson grass, produce a series of phytotoxic sesterterpenoids. These sesterterpenoids belong to the ophiobolin family. One of them, ophiobolin I, was characterized by x-ray diffraction and served as a crucial reference compound for characterizing four other ophiobolins. All of the ophiobolins studied produce characteristic lesions on host plants at concentrations of 1 mM to 1 μM. The ophiobolin characterized as 6-epiophiobolin A is selectively toxic to corn bearing Texas-male-sterile (Tms) cytoplasm when assayed in a dark CO_2 fixation assay. It is plausible that these ophiobolins had a role in the 1970 corn-blight epidemic in North America.**

In the summer of 1970, the corn crops of the United States and Canada were devastated by a corn-blight epidemic (1). This epidemic caused the greatest crop loss in the shortest time span of any plant disease ever reported. Midwest crop losses were 15% of the expected harvest, and in the Southeast losses were close to 50%. Corn plants with Texas-male-sterile (Tms) cytoplasm, which was most of the corn planted, were especially vulnerable. The causative agent of the epidemic was quickly identified as the fungus *Drechslera maydis* (also called *Helminthosporium maydis* or, in the perfect stage, *Cochliobolus heterostrophus*).

Fungal pathogens of plants often produce disease symptoms by elaborating one or more toxic compounds—phytotoxins. These phytotoxins can be specific for one species of plant or even for one strain of a given species and are called host-specific or host-selective phytotoxins. The phytotoxins produced by *D. maydis* have been investigated, but studies of their chemical identity have been contradictory. Some workers have reported partially characterized terpenes as selective phytotoxins for corn bearing Tms cytoplasm (2). Other workers suggested that linear polyketopolyalcohols were involved in the disease (3). A review has summarized these and other studies (4).

We became interested in fungal pathogens of weeds as a way of learning about the host selectivity of phytotoxins and developing models for additional herbicides (5). We elected to study *Drechslera sorghicola* (Lefebre and Sherwin) = *Helminthosporium sorghicola* because it caused leaf spots on Johnson grass (*Sorghum halapense* L.), a serious weed in all tropical and semitropical areas of the world. While exploring the chemistry and biology of several phytotoxic sesterterpenoids from this fungus, we learned that the perfect (sexual) stages of *D. maydis* (Nisik) = *H. maydis* (Nisik) and *D. sorghicola* are genetically compatible (R. R. Nelson, personal communication). This led us to examine the phytotoxins produced by each of these fungi, along with their biological activity on corn bearing Tms cytoplasm. Our findings on a series of phytotoxins are given below.

MATERIALS AND METHODS

D. maydis races T (isolate C4) and 0 (isolate Aus20239A3) were obtained from O. Yoder (Cornell University). *D. sorghicola* was purchased from the American Type Culture Collection (ATCC no. 28736). These fungi, maintained on potato dextrose/agar plates containing 18% (vol/vol) V-8 juice by volume, were used for toxin production by growing each of them in a New Brunswick incubator in 1 liter of modified M-1-D medium (2, 6), shaking at 200 rpm, illuminating, and maintaining a temperature of 26°C (7). Plants used in this study were grown under controlled environmental conditions of 12 hr of darkness at 22°C and 12 hr of light at 27°C with a light intensity of 170 $\mu E \cdot m^{-2}$. Corn (B37, Tms and N cytoplasms) was obtained from D. Mathre (Montana State University), and corn (W46A, Tms and N cytoplasms) was provided by V. Gracen (Cornell University). Sorghum species were available from the Montana State University collection.

Leaf Assay. A simple leaf-puncture assay was used as a rapid guide in isolating the suspected phytotoxins (5). The test material was applied in 2% (vol/vol) ethanol and a 1- to 5-μl droplet was placed on a leaf blade that was subsequently incubated in a sealed Petri dish containing moistened filter paper. The symptoms that developed within 24–48 hr depended upon the plants and varied from little or no reaction, to brownish, zonate spots, to spreading necrotic lesions. The extent of leaf tissue affected was estimated and used as a comparison among plant genotypes and test compounds. In no cases were symptoms observed with 2% (vol/vol) ethanol control solutions.

Root Cap Cell Assay. Root cap cells of corn (B37 with N and Tms cytoplasms) were prepared according to the method of Hawes (8). Toxin preparations were dissolved in 2% (vol/vol) ethanol (50 μl) and placed onto 50 μl of a cell suspension (A_{620}, 0.015). Following incubation at 27°C for 4 hr, 50 μl of 24 μM fluorescein diacetate was added. Microtest plates were scored according to the method of Strange *et al.* (9).

Mitochondria Assays. Washed mitochondria were isolated from the mesocotyls of 4-day-old etiolated B37 corn seedlings with Tms or N cytoplasm following the procedure of Siedow and Bickett (10). The electron transfer activity in the isolated mitochondria was measured on a Clark-type oxygen electrode and found to have respiratory control values during

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Abbreviation: Tms, Texas-male-sterile.

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succinate oxidation of 1.7 or 1.9 for mitochondria from Tms or N cytoplasm, respectively.

Mitochondrial swelling and electron transfer assays were as described by Klein and Koeppe (11). The reaction buffer for the swelling assays contained 300 mM sucrose, bovine serum albumin at 1 mg/ml, 20 mM Tris·HCl, and 0.2–0.25 mg of mitochondrial protein in a total volume 2.0 ml at pH 7.4. Additions were made as follows: 1 mM NADH, 3 mM methomyl, and 500 μM KCN. The spectrophotometric assays were at 520 nm and 25°C.

CO_2 Fixation Assays. Corn leaves of B37 and W64A with Tms or N cytoplasm were used in a CO_2 fixation assay carried out by constructing a dose–response curve following the method of Bhullar *et al.* (12). A 4-hr exposure at 26°C in light at 170 $\mu E \cdot m^{-2} \cdot s^{-1}$ was used. This test was repeated at least three times and yielded standard deviations of 14–60% of the mean, which is in close agreement with the variance usually reported for this test.

RESULTS

Phytotoxic Sesterterpenes. A series of phytotoxic sesterterpenoids was consistently isolated from 2-week-old cultures of *D. sorghicola* and *D. maydis*. The toxins were isolated by filtering the culture broth through four layers of cheesecloth (7). The filtrate was concentrated to one-third its original volume by rotary evaporation at 40°C and extracted three times with 200 ml of ethyl acetate. The remaining filtrate was also extracted with *n*-butanol, but this extract was devoid of biological activity. The combined ethyl acetate fractions were washed with 200 ml of water and evaporated to dryness at 30°C under reduced pressure. All of the detectable biological activity was in this residue. The residue was flash-chromatographed (Merck silica gel 60, 230–400 mesh, 30 g) using $CHCl_3$/MeOH, 20:1 (vol/vol). Fractions of 5 ml were collected, and fractions 12–16 showed activity in the leaf-puncture assay on all test plants. These fractions were further purified by preparative TLC (precoated TLC plates purchased from Merck, silica gel 60, F-254, 0.5 mm) using $CHCl_3$/MeOH, 14:1 (vol/vol), and toluene/ethyl acetate, 1:2 (vol/vol). Compounds were detected on TLC plates with anisaldehyde/sulfuric acid reagent (0.5% anisaldehyde and 1% sulfuric acid in 50 ml of acetic acid). The biologically active bands were chromatographed on a Pharmacia LH-20 (20 g) column eluted with MeOH to remove olefinic, inactive compounds. The purity of the compounds was determined by analytical HPLC using a Merck reverse phase RP-18 column (4.6 × 250 mm) eluted with CH_3CN/H_2O, 65:35 (vol/vol) at a flow rate of 1.0 ml/min and monitored with a 254-nm UV detector.

The active compounds isolated by this procedure were identified as ophiobolins by spectroscopic procedures. Their abbreviated names, retention times, and R_f values are given in Table 1.

Table 1. Retention times and R_f values of ophiobolins found in the culture fluids of *D. maydis* and *D. sorghicola*

Compound	R_T, min	R_f, System A	R_f, System B
Ophiobolin I (**1**)	10.9	0.33	0.45
Ophiobolin A (**2**)	8.8	0.52	0.67
Ophiobolin C (**3**)	30.5	0.60	0.82
25-Hydroxyophiobolin I (**4**)	4.3	0.08	0.20
6-Epianhydroophiobolin A (**5**)	11.8	0.53	0.68
6-Epiophiobolin A (**6**)	8.1	0.52	0.48

R_T were from an HPLC column. The HPLC system used a 4.6 × 250 mm Merck RP-18 column eluted with CH_3CN/H_2O, 65:35 (vol/vol) at a flow rate of 1.0 ml/min. R_f was determined by TLC. System A was $CHCl_3$/MeOH, 14:1 (vol/vol) and system B was toluene/ethyl acetate, 1:2 (vol/vol).

The structural analysis of the series began with ophiobolin I (**1**). The natural compound (1.2 mg) was converted to the *p*-bromobenzoate by dissolving it in 1.0 ml of dry pyridine and adding 3.0 mg of *p*-bromobenzoyl chloride. After a standard workup, the residue was chromatographed on preparative TLC plates with $CHCl_3$/MeOH, 50:1 (vol/vol) and toluene/ethyl acetate, 9:1 (vol/vol). This gave 2.0 mg of a crystalline derivative, mp 215–217°C. This derivative was characterized by single-crystal x-ray diffraction (5). Crystals formed in the monoclinic space group $P2_1$ with a = 13.759(12), b = 6.445(8), c = 16.655(13) Å, and β = 92.07(7)° with one molecule of composition $C_{32}H_{39}O_4Br$ in the asymmetric unit. All unique diffraction maxima with $2\theta < 114°$ were collected using graphite monochromated CuK$\bar{\alpha}$ radiation (1.54178 Å) and variable speed, 1° ω-scans. Only 1094 of the 1699 reflections collected in this fashion (64%) were judged observed [$F_o > 3\sigma(F_o)$] after correction for Lorentz, polarization, and background effects. A phasing model was found easily. Block diagonal least squares refinements with anisotropic heavy atoms and fixed hydrogens have converged to a current residual of 0.085 for the observed reflections. A computer generated drawing of the final x-ray model for the derivative is given in Fig. 1 and a chemical drawing of ophiobolin I is given as structure **1**. The absolute configuration was set from earlier studies on the ophiobolins, and the standard ophiobolin numbering scheme was used (13).

The important point about this structure was that the stereochemistry at C6 was opposite to that usually observed in the ophiobolins. The *cis*-ring fusion at C2 and C6 is the normal one, although 6-epiophiobolins have been characterized (14). To illustrate the standard stereochemistry, ophiobolin A, the first ophiobolin to be characterized, has structure **2**, and ophiobolin C is structure **3**. Securing the structure of ophiobolin I (**1**) allowed the structures of several other ophiobolins to be deduced. Table 2 contains selected ^1H NMR data for these ophiobolins. The structure of 25-hydroxyophiobolin I is shown as structure **4**. Its ^1H NMR spectrum clearly showed that either the C24 or the C25 methyl had been oxidized to the corresponding primary alcohol. The dramatic change in the chemical shift of the C18 proton from δ5.14 to δ5.43 indicated that it was the methyl *cis* to this proton that had been oxidized. The structure of 6-epianhydroophiobolin A (21-dehydroophiobolin I) is shown as structure **5**. The pair of protons associated with the primary alcohol at C21 in structure **1** (doublets at δ3.91 and δ4.16) are replaced by a singlet at δ9.24. 6-Epianhydroophiobolin A had earlier been isolated by Kim *et al.* (15) and by Canales (16) and tentatively characterized by ^1H and ^{13}C NMR spectroscopy. The final epiophiobolin to be isolated was 6-epiophiobolin A (3-hydroxy-21-dehydroophiobolin I) shown as structure **6**. The crucial experiment in the characterization of this compound was the observation that it was not ophiobolin A (**2**), but could be converted to ophiobolin A with aqueous dimethyl sulfoxide (15). When the conversion was done in a deuterated solvent, the proton at C6 in the resulting ophiobolin A (**2**) was completely exchanged. There was also a nuclear Overhauser enhancement of the signal of H10 upon irradiation of the resonance for H6 (16). The characterized ophiobolin A (**2**) was produced by *D. maydis* and *D. sorghicola*, and *D. maydis* produced ophiobolin C (**3**). The optical rotations were as follows: structure **1**, $[\alpha]_D^{22}$+48° (*c* 3.8, $CHCl_3$); structure **4**, $[\alpha]_D^{22}$+22° (*c* 1.6, $CHCl_3$); and structure **6**, $[\alpha]_D^{22}$+46° (*c* 5.3, $CHCl_3$).

Ophiobolins in Culture. The six ophiobolins discussed above were examined both quantitatively and qualitatively from *D. maydis* races T and O and from *D. sorghicola*. All isolated ophiobolins were characterized by ^1H NMR spectroscopy and MS to insure their identity. After HPLC separation, a quantitative estimation was made for each of

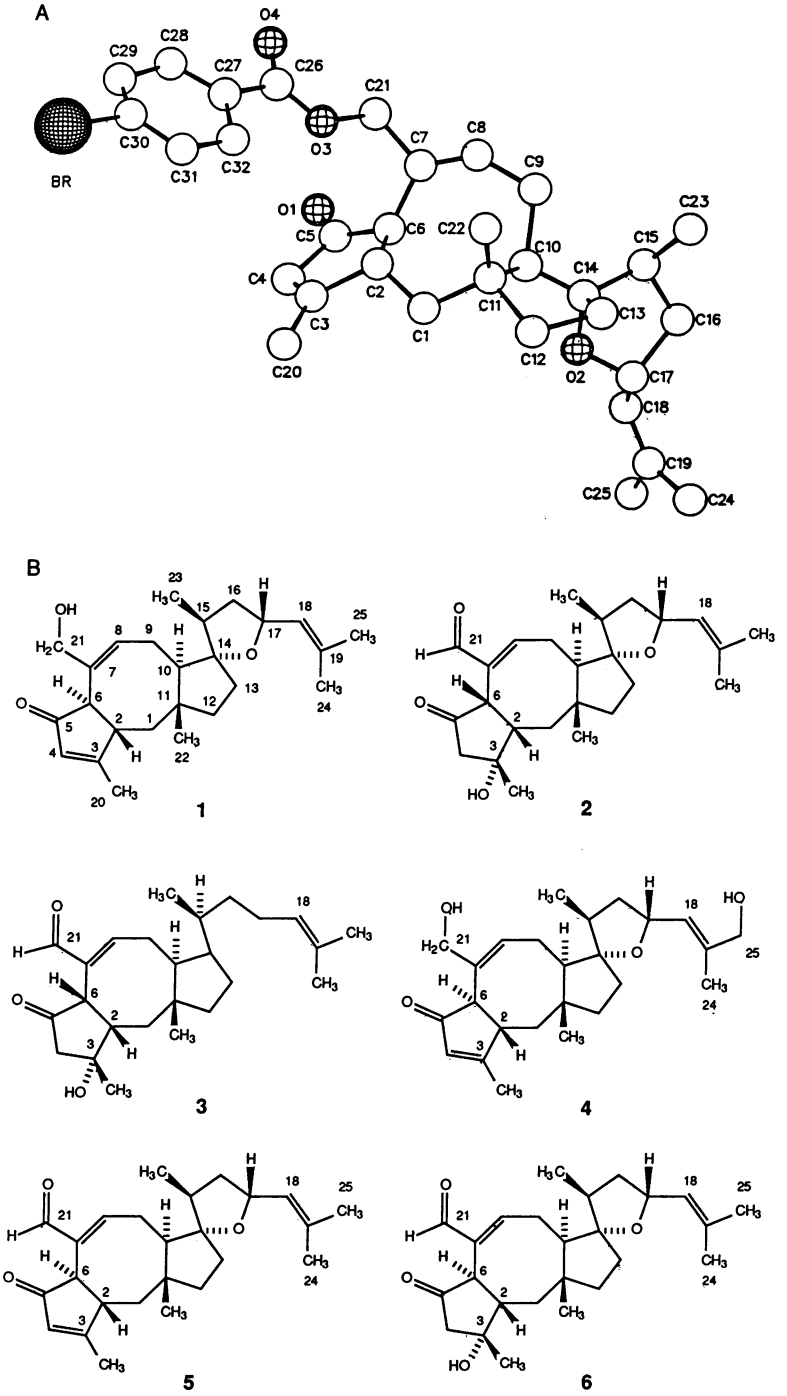

FIG. 1. (*A*) A computer generated perspective drawing of the final x-ray model of the *p*-bromobenzoate derivative of ophiobolin I. Hydrogens are omitted for clarity, and the absolute configuration was set from earlier studies on the ophiobolins (*B*).

the ophiobolins based on the dry weight of the fungus. These results are given in Table 3. The maximum production of these compounds occurred after 14 days of incubation, and one compound, 6-epiophiobolin A (**6**), was not detected until day 10. If cultures were incubated over 3 weeks, the extracts were no longer completely separable under our HPLC conditions.

As can be seen in Table 3, there was a noticeable difference in the quality and quantity of ophiobolins in *D. sorghicola* in comparison to *D. maydis* races T and O. For example, 25-hydroxyophiobolin I (**4**) was a major metabolite in *D. sorghicola*, but occurred only in minor amounts in *D. maydis* race O. Ophiobolin C occurred in roughly equal amounts in both races of *D. maydis*, but was not detected in *D. sorghicola*. Finally, race T of *D. maydis* made five times more 25-hydroxyophiobolin I (**4**) than race O.

Plant Assays. Since a number of phytotoxic effects had been attributed to *D. maydis* extracts, it was crucial to test the isolated compounds in a variety of assays. All of the ophiobolins discussed in this report were tested by the leaf puncture method on both Tms and N corn (B37 and W64A genetic backgrounds), Johnson grass, and sorghum. All were active, and a summary of the activity is given in Table 4. At 1 mM, they were all capable of causing brownish lesions. However, the most bioactive compound was 6-epiophiobolin A (**6**), which produced large lesions with reddish runners on

Table 2. Summary of proton NMR data for the ophiobolins isolated from *Drechslera spp*

	Ophiobolin I (1)	25-Hydroxyophiobolin I (4)	6-Epianhydroophiobolin A (5)	6-Epiophiobolin A (6)
H2	2.53 m	2.54 m	2.30 m	2.11 m
H4	5.96 d 1.1	5.96 s	6.04 s	2.41 d 16.7
H4				3.06 d 16.7
H6	3.67 d 2.6	3.62 d 2.6	3.43 d 4.1	3.36 d 10.5
H8	5.78 d 3.8	5.77 d 4.0	6.82 dd 2.3, 6.6	6.88 d 2.0, 6.8
H17	4.58 dd 7.2, 15.8	4.63 dd 7.2, 15.4	4.60 dd 7.1, 15.7	4.62 dd 8.5, 15.6
H18	5.14 ddd 1.0, 1.0, 7.2	5.43 dd 0.8, 7.2	5.14 ddd 1.0, 1.0, 7.2	5.14 ddd 1.0, 1.0, 7.2
H20	2.08 s	2.08 s	2.05 s	1.42 s
H21	3.91 d 11.0	3.17 d 11.9	9.32 s	9.22 s
H21	4.16 d 11.0	3.91 d 11.9		
H22	1.00 s	0.99 s	0.88 s	0.85 s
H23	1.02 d 6.9	1.03 d 6.9	1.05 d 7.0	1.04 d 6.8
H24	1.70 d 1.0	1.69 d 0.8	1.71 d 1.0	1.71 d 1.0
H25	1.66 d 1.0	4.00 s	1.66 d 1.0	1.67 d 1.0

The proton assignments are given on the left. Each entry is the chemical shift (δ), multiplicity, and coupling constant(s) (Hz).

Tms corn. These appeared to be very similar to lesions found on diseased corn in the field. On N corn, 6-epiophiobolin A produced only sunken lesions similar in size to those on Tms corn but lacking colored runners. Furthermore, Tms corn with a W64A genetic background appeared to be more sensitive than Tms with a B37 background, which agrees with the field observations of Lim (17). In contrast to all of the other compounds, 6-epiophiobolin A (**6**) produced noticeable lesions on both Tms and N corn at micromolar concentrations.

All ophiobolins were tested on isolated root cap cells of both B37 N and Tms corn. All produced 50% cell death between 0.18 and 0.60 mM; however, in this assay, 6-epianhydroophiobolin A (**5**) was significantly more effective (0.18 mM) than any of the others ($P < 0.05$). There were no differences in response between corn with Tms and N cytoplasm. These experiments were repeated three times, and the data were analyzed according to Student's *t* test. The least effective compounds in this assay were ophiobolin I (**1**) and 25-hydroxyophiobolin I (**4**) that required at least 0.60 mM to cause 50% cell death. All of the other compounds were effective in the 0.30 to 0.38 mM range.

Since ophiobolin A as well as crude toxin preparations from *D. maydis* were known to inhibit dark CO_2 fixation, we tested each of the purified ophiobolins in this assay. These results are given in Table 5. As in the leaf puncture assay, 6-epiophiobolin A (**6**) was the most effective compound in disrupting dark CO_2 fixation in Tms corn. The effect was much more pronounced in corn with a W64A nuclear background. Comparable tests with the other ophiobolins, with the exception of ophiobolin C (**3**), generally showed a tendency toward greater sensitivity of corn with Tms cytoplasm than N cytoplasm (Table 5). Crude toxin preparations, the ethyl acetate fraction, at concentrations up to 100 μg/ml did not cause any greater reduction in CO_2 fixation than a combination of purified ophiobolins in their naturally occurring *D. maydis* ratios at the same concentration.

Since crude culture filtrates of *D. maydis* have traditionally been noted to affect mitochondrial activities in corn bearing

Table 3. Quantitative and qualitative determinations of the ophiobolins present in 2-week-old culture fluids of *D. maydis* and *D. sorghicola*

	Ophiobolin concentration, mmol/g (dry weight)		
	D. maydis		
Ophiobolin	Race T	Race O	*D. sorghicola*
Ophiobolin I (**1**)	0.3	0.7	0.5
25-Hydroxyophiobolin I (**4**)	0.3	0.06	3.1
6-Epianhydroophiobolin A (**5**)	Trace	Trace	Not detected
6-Epiophiobolin A (**6**)	1.3	1.5	1.7
Ophiobolin A (**2**)	8.0	8.4	5.2
Ophiobolin C (**3**)	1.4	1.7	Not detected

Concentrations are in mmol/g for the dry weight of the fungus. All experiments were carried out at least twice with a variation not exceeding 10% of the average value recorded.

Table 4. Effects of the ophiobolins from *D. maydis* and *D. sorghicola* in the leaf puncture wound test on corn (*Zea mays*), Johnson grass (*Sorghum halepense*), and sorghum (*Sorghum bicolor*)

		Lesion size*			
		Corn (W64A and B37)			
Ophiobolin structure	Concentration, M	T corn	N corn	Johnson grass	Sorghum
1	10^{-3}	+	+	+	+
	10^{-4}	+	+	+	+
	10^{-5}	−	−	+	+
	10^{-6}	−	−	−	−
4	10^{-3}	+	+	+	+
	10^{-4}	−	−	−	+
	10^{-5}	−	−	−	−
	10^{-6}	−	−	−	−
5	10^{-3}	+	+	+	+
	10^{-4}	+	+	−	−
	10^{-5}	−	−	−	−
	10^{-6}	−	−	−	−
6	10^{-3}	+++†	+++‡	+++§	++¶
	10^{-4}	+	++	+++	++
	10^{-5}	+	+	++	+
	10^{-6}	+	+	−	−
2	10^{-3}	++	++	++	++
	10^{-4}	+	+	++	++
	10^{-5}	−	−	−	+
	10^{-6}	−	−	−	−
3	10^{-3}	+	+	+	+
	10^{-4}	+	+	−	+
	10^{-5}	−	−	−	−
	10^{-6}	−	−	−	−

*Lesion size: +++, >5 × 3 mm; ++, 2 × 2 to 5 × 3 mm; +, 0.5 × 0.5 to 2 × 2 mm; −, <0.5 × 0.5 mm.
†Reddish-brown lesion with several 5-mm reddish runners. The runners were more apparent in W64A-Tms corn than in B37-Tms corn.
‡Sunken lesion.
§Necrotic lesion with brown runner.
¶Dark, necrotic lesion.

Table 5. Dark CO_2 fixation in corn B37 and W64A (N and T cytoplasm) by various ophiobolins isolated from *D. maydis* and *D. sorghicola*

Ophiobolin structure	IC_{50}, mM			
	W64A		B37	
	T	N	T	N
6	0.8	70	21	115
5	10	55	52	65
1	13	23	137	105
4	100	262	325	325
2	62	95	75	80
3	175	157	187	225

The data are reported as concentration (μM) of compound required to produce a 50% inhibition of dark CO_2 fixation in corn leaf pieces.

Tms cytoplasm, we tested each of the compounds, except for ophiobolin C, for stimulation of mitochondrial swelling and NADH oxidation, and inhibition of malate oxidation. None of the compounds stimulated swelling in mitochondria from either Tms or N corn (W64A or B37) at concentrations between 50 and 125 μM. There was also no effect on NADH or malate oxidation in the mitochondria of Tms or N corn. Canales (16) did report mitochondrial activity with structures **5** and **6** using a different assay system. Finally, Leung *et al.* (18) noted activity but no selectivity in N and Tms corn with structures **2**, **5**, and **6** in an ion leakage assay.

DISCUSSION

In limited greenhouse tests, *D. maydis* races T and O, as well as *D. sorghicola*, were pathogenic to corn (W64A and B37) carrying both Tms and N cytoplasm. However, *D. maydis* race T is much more virulent to corn under field conditions than is race O (1, 19). Based on this study, one factor that may contribute to the increased pathogenicity of *D. maydis* race T is its ability to produce more 25-hydroxyophiobolin I (**4**) than race O (Table 3). It is also conceivable that the regulation of toxin production by one or more host factors may contribute to the virulence of the organisms (6).

The biological relationship between *D. sorghicola* and *D. maydis* is interesting from a pathological point of view because they differ in the ophiobolins that they produce (Table 3). Since both are sexually compatible, it is possible that *D. maydis*, a pathogen normally found on corn, may have exchanged genetic information with *D. sorghicola* leading to the production of compounds in the ophiobolin series. It appears that genetic studies, using these ophiobolins as markers in combination with observations on the pathogenicity of the offspring, are warranted. Further, we feel that this work shows the importance of examining the bioactive secondary metabolites of fungi that attack weeds, since their progeny may develop into new pathogens of crop plants.

Canales (16), relying primarily on NMR techniques, showed the presence of 6-epiophiobolin A (**6**) and 6-epianhydroophiobolin A (**5**) in cultures of *D. maydis*. He also indicated that these compounds possessed selective activity to Tms corn. This report confirms his structural assignments by x-ray diffraction and spectroscopic analysis and augments the family of characterized ophiobolins. To our knowledge, ophiobolin I and 25-hydroxyophiobolin I (**4**) have not been previously reported.

It appears that more than one biological function is involved in the mode of action of these ophiobolins, since, in the various bioassays, different compounds appeared to be the most active. For instance, the most active compound in the dark CO_2 fixation and leaf bioassays was 6-epiophiobolin A (**6**), while in the root cap cell assay, 6-epianhydroophiobolin A (**5**) was the most active.

Interestingly, none of the ophiobolins tested showed activity in mitochondrial assays, yet toxin preparations from *D. maydis* reported by others have this activity (16, 20, 21, 22). Based on this study, it is apparent that there is more than one factor in *D. maydis* and *D. sorghicola* that has the potential of contributing to their pathogenicity on corn and sorghum, respectively. Some of the members of the ophiobolin series have some selectivity for corn with Tms cytoplasm (Tables 4 and 5), but the activity seems to be more directed toward the chloroplast or whole-cell function than to the mitochondrion. On the other hand, ophiobolin A described from *D. maydis* does not seem to discriminate between Tms and N corn (23). It is phytotoxic to both. Relatively high levels of ophiobolin A (**2**) or C (**3**) are required to be effective (Tables 4 and 5). The present report certainly does not rule out the possibility of still other compounds being produced by *D. maydis* and *D. sorghicola* that have selective phytotoxic activity (3, 22).

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