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3-ANHYDROOPHIOBOLIN A AND 3-ANHYDRO-6-*EPI*-OPHIOBOLIN A, PHYTOTOXIC METABOLITES OF THE JOHNSON GRASS PATHOGEN *BIPOLARIS SORGHICOLA*[1]

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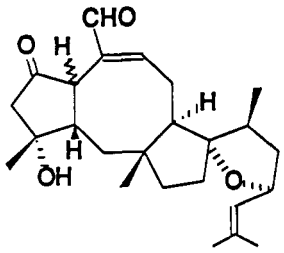
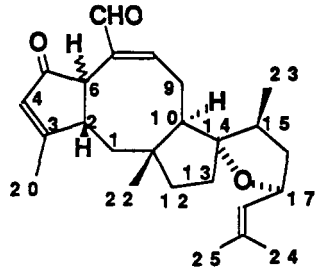
ABSTRACT.—Culture filtrates of the Johnson grass pathogen *Bipolaris sorghicola* contain 3-anhydroophiobolin A [**3**] and 3-anhydro-6-*epi*-ophiobolin A [**4**] in addition to the major phytotoxins ophiobolin A [**1**] and 6-*epi*-ophiobolin A [**2**].

As part of our research on phytotoxins produced by weed pathogens (1,2) we have investigated the production of phytotoxic metabolites by *Bipolaris sorghicola* [(Lefebvre and Sherwin) Alcorn] (Poaceae), a fungal pathogen of Johnson grass [*Sorghum halepense* (L.) Pers.]. Use of the leaf spot assay with sorghum [*Sorghum bicolor* (L.) Moench] as the test plant allowed us to isolate four phytotoxic metabolites from the organic crude extract of the fungal culture filtrate. The phytotoxins were identified as ophiobolin A [**1**], 6-*epi*-ophiobolin A [**2**], 3-anhydroophiobolin A [**3**], and 3-anhydro-6-*epi*-ophiobolin A [**4**]. Ophiobolin A and 6-*epi*-ophiobolin A have been reported previously from *Drechslera sorghicola* (=*Bipolaris sorghicola*), but 3-anhydro-6-*epi*-ophiobolin A was not previously detected in quantitative analysis of two-week-old culture fluid by hplc (3). Anhydro-6-*epi*-ophiobolin A has been reported previously from cultures of *Drechslera maydis* (3,4), *Drechslera oryzae* (5), and *Helminthosporium* sp. (6). The unepimerized 3-anhydroophiobolin A has been previously found only once in the culture filtrate of *Helminthosporium* sp. (6).



1 A/B cis
2 A/B trans



3 A/B cis
4 A/B trans

Ophiobolin A was first reported as having a series of damaging effects against rice plants (7), and all four ophiobolins were reported as inhibitors of photosynthesis (6). Recently 6-*epi*-ophiobolin A has been reported as the most active metabolite when tested by both a dark CO_2 fixation assay and leaf spot assays using maize, Johnson grass, sorghum, and rice (3,5). Both 6-*epi*-ophiobolin A and 3-anhydro-6-*epi*-ophiobolin A have been implicated as selective toxins of *D. maydis* race T, a pathogen of maize containing T-cytoplasm (3,4).

Using a leaf spot assay we have evaluated the phytotoxicity of ophiobo-

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lins **1–4** against several plants on the basis of the area of necrosis. The lesions observed on sorghum, maize, and bentgrass (*Agrostis alba* L.) were all similar to those caused by the fungus on Johnson grass in the field, namely, reddish brown necrotic area limited by a dark brown border. In sicklepod (*Cassia obtusifolia* L.) and morning glory [*Ipomoea purpurea* (L.) Roth], the toxic effects appeared in the form of black necrotic spots. At high concentrations the necrotic spots in sorghum, maize, bentgrass, and sicklepod were surrounded by a chlorotic zone, whereas in morning glory veinal chlorosis was observed. The results of our tests are summarized in Table 1. Ophiobolin A and 6-*epi*-ophiobolin A were more phytotoxic than their anhydro derivatives against sorghum, sicklepod, and maize (variety A632). Epiophiobolin A at high concentrations produced the largest lesions on leaves of all plants tested except morning glory. The anhydro derivatives were generally less phytotoxic and not toxic at all to morning glory leaves, even at concentrations of 2 mg/ml.

EXPERIMENTAL

General experimental details and the leaf spot assay have been described previously (1). Necrotic areas were measured after 72 h. All tlc were developed on Merck Si gel 60 with solvent A [CH_2Cl_2-Me_2CO (9:1)] and solvent B [Et_2O-C_6H_6 (3:2)]. Mass spectra and nmr consistent with published data were obtained for all compounds.

CULTURE OF *B. SORGHICOLA* AND FRACTIONATION OF CULTURE FILTRATE.—A culture of *B. sorghicola* was obtained from the collection of Dr. Kurt Leonard, Dept. of Plant Pathology, North Carolina State University (collection no. 2-3T1-97). The fungus was maintained and cultured as described (1). Following removal of the mycelial mat, the culture filtrate was extracted three times with equal amounts of EtOAc. Removal of the solvent in vacuo yielded the crude extract (ca. 140–155 mg/liter). Purification of the crude extract (714 mg) was carried out using flash cc (5 × 16 cm column, Merck Si gel 60). Stepwise elution with C_6H_6-Me_2CO (95:5 to 80:20) yielded fractions A–J. Leaf spot assay showed that

TABLE 1. Necrotic Area (mm^2) Caused by Ophiobolins in Leaf Spot Test.

Metabolite	Drop (μg/5 μl)	Sorghum	Sickle-pod	Maize	Morning glory	Bent-grass
Ophiobolin A [**1**]	10	18	16	24	14	15
	5	14	8	20	9	10
	2.5	13	8	10	6	9
	1	12	6	NT	NT	NT
	0.5	5	6	NT	NT	NT
	0.25	6	6	NT	NT	NT
6-*epi*-Ophiobolin A [**2**]	10	30	24	37	9	43
	5	20	15	35	5	19
	2.5	17	11	28	2	8
	1	7	6	NT	NT	NT
	0.5	2	4	NT	NT	NT
	0.25	1	1	NT	NT	NT
3-Anhydroophiobolin A [**3**]	10	15	14	25	0	11
	5	14	11	21	0	10
	2.5	11	8	19	0	5
	1	6	3	NT	NT	NT
	0.5	2	0	NT	NT	NT
	0.25	0	0	NT	NT	NT
3-Anhydro-6-*epi*-ophiobolin A [**4**]	10	14	17	24	0	10
	5	10	13	21	0	8
	2.5	10	9	18	0	9
	1	1	1	NT	NT	NT
	0.5	0	0	NT	NT	NT
	0.25	0	0	NT	NT	NT

fractions B, C, and F possessed the strongest phytotoxic activity.

ISOLATION OF OPHIOBOLIN A [**1**].—Fraction D (152.6 mg) contained a single major component that was obtained as short needles (48.2 mg) by recrystallization from Et_2O: mp 180 [lit. (4) ophiobolin A mp 181], eims *m/z* $[M]^+$ 400 (30%), 382 (30), 165 (100); tlc R_f solvent A 0.49, solvent B 0.40. Comparison of R_f values and spectroscopic data (uv, ir, ms, and ^1H nmr) of this metabolite with those reported (8,9) confirmed its identity as ophiobolin A [**1**].

ISOLATION OF 6-*EPI*-OPHIOBOLIN A [**2**].—The major metabolite present in fraction F (29.1 mg) was obtained in pure form (11.4 mg) by preparative tlc purification [C_6H_6-Me_2CO (75:25)] of the mixture. The pure metabolite migrated at R_f 0.31 on tlc in solvent A and R_f 0.32 in solvent B. Direct comparison of the spectroscopic data of this metabolite with that of **1**, as well as with spectroscopic data for **2** in the literature (3), resulted in its identification as 6-*epi*-ophiobolin A [**2**].

ISOLATION OF 3-ANHYDROOPHIOBOLIN A [**3**] AND 3-ANHYDRO-6-*EPI*-OPHIOBOLIN A [**4**].—Tlc analysis of fraction B (44.2 mg) showed the presence of two rather nonpolar compounds. Each component was obtained pure by preparative tlc [Et_2O-C_6H_6 (3:2) 2×]. The more polar compound (tlc R_f 0.64 in solvent A, 0.40 in solvent B) was observed to undergo conversion to the less polar one (tlc R_f 0.69 in solvent A, 0.57 in solvent B) on long storage. The mass spectrum of the more stable, less polar isomer indicated an anhydroophiobolin ($[M]^+$ 382). The ^1H nmr of the less polar isomer was identical to that reported for 3-anhydro-6-*epi*-ophiobolin A [**4**].

The less stable, more polar isomer was identified as 3-anhydroophiobolin A by comparison to published spectra (6). Additional proton assignments were made by comparison with the nmr spectra of the other three ophiobolins isolated. ^1H nmr (250 mHz) 9.34 (s, CHO), 7.05 (dd, 4.8, 7.8, H-8), 6.11 (t, <1, H-4b), 5.13 (dt, 1.3, 8.2, H-18), 4.43 (q, 7.5, H-17), 3.84 (d, 7.2, H-6), 3.13 (br t, H-2), 2.52 (ddd, 3, 4.4, 16.9, H-9a), 2.4 (m, H-10), 2.3 (m, H-9b), 2.12 (s, H-20), 1.69 (s, H-25), 1.62 (s, H-24), 1.02 (d, 7, H-23), 0.73 (s, H-22).

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