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VICTOXININE AND PREHELMINTHOSPOROLACTONE, TWO MINOR PHYTOTOXIC METABOLITES PRODUCED BY *BIPOLARIS* SP., A PATHOGEN OF JOHNSON GRASS[1,2]

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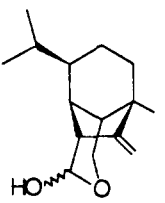
ABSTRACT.—Two additional phytotoxins produced by *Bipolaris* sp. strain 36, a pathogen of Johnson grass, have been identified as victoxinine [**3**] and prehelminthosporolactone [**4**].

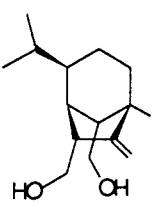
We have recently described the isolation and identification of prehelminthosporol [**1**] and dihydroprehelminthosporol [**2**] as phytotoxins produced by *Bipolaris* sp. strain 36 (ATCC 64838), a fungal pathogen of Johnson grass [*Sorghum halepense* (L.) Pers.] (1). While scaling up production of these phytotoxins we have isolated two minor phytotoxic metabolites identified as victoxinine [**3**] and prehelminthosporolactone [**4**].

Culturing of the pathogen and initial purification of the crude extract were carried out as described previously (1). Fractions containing **2** also showed the presence of a white spot against a light blue background on tlc plates treated with phosphomolybdic acid. Purification of the mixture by flash cc yielded each component in pure form. The metabolite that does not react with phosphomolybdic acid was identified as victoxinine [**3**], a nitrogen-containing terpenoid first isolated from culture filtrates of *Helminthosporium victoriae* (2), the causal agent of Victoria blight of oats. Victoxinine has been reported from filtrates of *Helminthosporium sativum* (reclassified as *Bipolaris sorokiniana*) (3), as well as from pathogenic and non-pathogenic strains of *H. victoriae* (2,4,5). The structure of victoxinine was first elucidated by chemical correlation with **1** (3). A more recent report on the isolation of victoxinine-α-glycerophosphate from *H. victoriae* (6) provides ^{1}H and ^{13}C chemical shift data for **3** that are identical to our spectral data.

A further new metabolite was obtained as colorless needles after purification by flash cc of a fraction containing **1** as the major component. Both the molecular formula of $C_{15}H_{22}O_2$ and the spectroscopic data for this new metabolite were found to be identical to those reported for prehelminthosporolactone [**4**] obtained by oxidation of **1** (1,7,8). While the lactone is a possible product of air oxidation of **1**, its presence in culture filtrate at 48 h after inoculation



1



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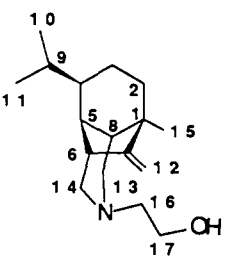

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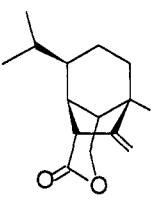

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[1]Dedicated to Prof. Kenneth L. Rinehart, Jr., School of Chemistry, University of Illinois, on the occasion of his 60th birthday.

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suggests that it is a metabolic product of *Bipolaris* sp. strain 36. This is the first report of the lactone as a naturally occurring substance.

Of the four metabolites mentioned, only victoxinine has been reported previously as having phytotoxic activity. The hydrochloride salt of victoxinine inhibited root growth of *H. victoriae* -susceptible and -resistant oat plants at 75 μg/ml (2). When tested by the root inhibition assay, victoxinine free base was found to be toxic to cereals in the following order: oats > rye and barley > wheat > sorghum (9). We have evaluated the phytotoxicity of the four metabolites against a number of plants using the leaf spot assay as described (1). The results of this evaluation, listed in Table 1, were determined by considering the time required for the test metabolite to cause a visible lesion and the extent of the lesion. Metabolites **1**, **2**, and **4** produced lesions on Johnson grass and sorghum resembling those observed in the fungal disease in the field: a reddish brown center surrounded by a black circle at the edge of the drop containing the phytotoxin, with an outer, chlorotic zone extending beyond the drop. In contrast, victoxinine did not provoke the reddening but gave a water-soaked translucent appearance with defined but irregular necrotic boundaries. The red wound response is caused by stress response metabolites of sorghum identified as 3-deoxyanthocyanidins (10). The wound-induced antifungal anthocyanidins have been characterized as phytoalexins of sorghum. The fungal phytotoxins **1**, **2**, and **4** are elicitors of much stronger reddening than caused by wounding alone, but victoxinine is a toxin which does not elicit this phytoalexin response in sorghum. In corn and bentgrass the effects caused by all four metabolites were similar in appearance: a light brown area surrounded by a chlorotic zone. Sicklepod and morning glory showed the phytotoxic effects of the different metabolites by the presence of necrotic lesions that at high concentrations extended beyond the area under the drop. Dihydroprehelminthosporol is the most toxic meta-

TABLE 1. Relative Leaf Spot Damage Caused by *Bipolaris* Metabolites on Sorghum (*Sorghum bicolor*), Sicklepod (*Cassia obtusifolia*), Maize (*Zea mays*), Morning Glory (*Ipomoea purpurea*), and Bentgrass (*Agrostis alba*).[a]

Metabolite	μg/5 ul drop	Sorghum	Sicklepod	Maize	Morning Glory	Bentgrass
Prehelminthosporol [**1**]	10	3	3	2	3	2
	5	2	2	1	1	1
	2.5	0	1	0	0	0
	1	0	0	NT	NT	NT
Dihydroprehelminthosporol [**2**]	10	5	4	4	1	4
	5	3	3	3	0	3
	2.5	0	2	1	0	1
	1	0	0	NT	NT	NT
Victoxinine [**3**]	10	3	3	2	1	2
	5	1	1	0	0	1
	2.5	0	1	0	0	0
	1	0	0	NT	NT	NT
Prehelminthosporolactone [**4**]	10	2	2	NT	NT	0
	5	0	1	NT	NT	0
	2.5	0	0	NT	NT	0
	1	0	0	NT	NT	NT

[a]0 = not toxic, 1–5 = increasing damage, NT = not tested.

bolite of *Bipolaris* sp. strain 36 against all of the plants tested except morning glory.

EXPERIMENTAL

The *Bipolaris* strain used in this and a previous study (1) was isolated from a Johnson grass leaf in Wake County, North Carolina (11). Culture of the fungus and initial purification of the organic extract of the culture filtrate have been described in the previous report (1). All tlc was conducted on E. Merck Si gel 60.

ISOLATION OF VICTOXININE [**3**].—Victoxinine was detected on tlc plates treated with phosphomolybdic acid as a white spot on a light blue background. Fractions containing dihydroprehelminthosporol and victoxinine (63 mg) were fractionated by flash cc (2 × 15 cm column, E. Merck Si gel 60). Stepwise elution with hexane-Et_2O (90:10 to 30:70) yielded each component in pure form. Victoxinine (7 mg) was identified by comparison of its ir, ^{1}H-, and ^{13}C-nmr spectra with data reported in the literature (3,6). Tlc R_f 0.14 [Et_2O-hexane (80:20)], 0.21 [C_6H_6-Me_2CO (70:30)]; ir (thin film on KBr) 3464, 3063, 1655 cm^{-1}; ^{1}H nmr (500 MHz, $CDCl_3$) 4.75 (1H, s, H-12a), 4.59 (1H, s, H-12b), 3.50 (2H, m, H-17), 2.99 (1H, dd, 3.0, 10.9 Hz, H-13a), 2.81 (1H, dd, 4.7, 9.3 Hz, H-14a), 2.58 (1H, br m, H-6), 2.52 (1H, ddd, 4.7, 8.0, 12.6 Hz, H-16a), 2.41 (1H, dt, 4.4, 12.5 Hz, H-16b), 2.34 (1H, dd, 1.6, 10.9 Hz, H-13b), 2.23 (1H, d, 9.4 Hz, H-14b), 1.82 (1H, br s, H-5), 1.42 (1H, m, H-8), 1.35 (1H, m, H-9), 1.13 (3H, s, H-15), 0.90 (3H, d, 6.6 Hz, H-10), 0.86 (3H, d, 6.6, H-11); couplings identified by 2D nmr $J_{5,8}$, $J_{6,8}$, $J_{6,14}$ all <1 Hz, $J_{6,14a}=4.7$, $J_{8,13a}=3.0$, $J_{8,13b}=1.6$, $J_{13a,b}=10.9$, $J_{14a,b}=9.3$, $J_{16a,b}=12.5$, $J_{16a,17a}=4.7$, $J_{16a,17b}=8.0$, $J_{16b,17a}=4.4$, $J_{16b,17b}=4.4$; cims (CH_4) *m/z* $[M]^+$ 264 (100%), 232 (39%); hrms (parent ion not observed) $[M-CH_2OH]^+$ calcd for $C_{16}H_{22}N$, 232.2065, found 232.2066.

Once the basic nature of this metabolite was recognized, improved yields of nearly pure victoxinine were obtained by acid-base extraction. The organic crude extract was suspended in H_2O-MeOH (9:1) (ca. 100 ml of solution/200 mg of crude). The resulting aqueous suspension (pH 3.8) was adjusted to pH 2 by adding 4 N HCl. Successive extractions with EtOAc (ratio of solvent to suspension 2:1, 1:1, 1:1) gave the neutral fraction. Following removal of excess EtOAc, the aqueous layer was readjusted to pH 12 by addition of 2.5 N NaOH. EtOAc extraction (same ratios as above) gave the basic fraction containing **3** as the major component.

ISOLATION OF PREHELMINTHOSPOROLACTONE [**4**].—A fraction (163.1 mg) containing **1** and an unidentified less polar compound was purified by flash cc (2 × 20 cm column) using C_6H_6-Et_2O (95:5) as the eluting solvent. The purified unknown metabolite was identical in all respects (tlc, ir, ^{1}H nmr, ms, and mp) with prehelminthosporolactone [**4**] obtained by oxidation of prehelminthosporol [**1**] (1,7,8).

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