Production of Amines Similar to Victoxinine by Helminthosporium carbonum

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Victoxinine ($C_{17}H_{27}NO$), a tricyclic secondary amine, was first identified as a part of the host-specific toxin (HV-toxin) of *Helminthosporium victoriae* Meehan & Murphy (3). Later, free victoxinine was found in culture filtrates of the same fungus (4). The presence of iso-propyl absorption in its infrared spectrum indicates that victoxinine is a sesquiterpene (Pringle, *unpublished data*).

Victoxinine could be a precursor in the biosynthesis of HV-toxin, as suggested by Nishimura et al. (2). If so, its unusual chemical constitution would make it a good marker for future studies, which could combine biochemical and genetic analyses. Some genetic data are available (1, 6); biochemical data to supplement them are now needed. However, a possible complication developed when another fungus, *H. carbonum* Ullstrup, was reported to produce victoxinine (2, 7).

Nishimura et al. (2) identified 135 isolates of several different *Helminthosporium* species and examined them for ability to produce victoxinine in culture. Survey methods included the use of ascending paper chromatography with *n*-propanol:acetic acid:water (200:3:100 v/v). Spots with the R_F of victoxinine were detected by spraying with iodine solution; total amine content in the spots was determined by the Sasaki-Fujimaki picrate method. All *H. victoriae* isolates and 5 of 30 isolates of *H. carbonum* (races 1 and 2) produced a substance that gave an iodine-positive spot with an R_F of 0.84, suggesting victoxinine. No other culture gave a positive test. Earlier, Pringle & Braun (unpublished data) also showed no victoxinine production by species other than *H. victoriae*. *H. carbonum* was not included in the earlier survey, which was designed to find a good source of victoxinine (4).

We have re-examined the five isolates of *H. carbonum* used by Nishimura et al. (2) for ability to produce victoxinine. Three of the isolates were race 1 from corn, and produced the toxin-specific to *H. carbonum*-susceptible corn (HC-toxin) (5). The other isolates apparently were *H. carbonum* race 2, since they did not produce HC-toxin. All isolates were supplied by R. R. Nelson, Penn. State Univ. (The source of each isolate is given in citation 2.)

Diethyl ether extracts of culture filtrates (100 ml) from each isolate were first prepared as described by Nishimura et al. (2). Later, in order to produce more sharply resolved chromatograms, duplicate 100-ml portions of filtrates were given a preliminary extraction with chloroform and *n*-butanol to remove interfering substances. After this treatment, the solutions were extracted with diethyl ether at pH 8.4 (with sodium bicarbonate). Each extract was evaporated to dryness under reduced pressure; residues were then dissolved in ethanol and chromatographed on paper with several solvent systems (Table 1).

All extracts from filtrates of the five *H. carbonum* isolates gave a poorly defined yellow-brown streak in the vicinity of the R_F of victoxinine (0.84) when chromatograms were developed by the ascending technique

TABLE 1. Paper chromatography of victoxinine and amines, extracted by ether at pH 8.4, from five isolates of *Helminthosporium carbonum* in several solvent systems

Solvent system	Victoxinine. HCl (20 µg/ml)	Isolate[a] 1	2	3	4	5
	R_F	R_F	R_F	R_F	R_F	R_F
A *n*-propanol:acetic acid:water (200:3:100 v/v) ascending	0.84 (compact)	0.84 (streak)	0.84 (streak)	0.84 (streak)	0.84 (streak)	0.84 (streak)
B *n*-propanol:acetic acid:water (200:3:100 v/v) descending	0.84	0.80[b]	0.80	0.80	0.82	0.81
		0.85	0.84	0.85	0.85	0.85
		0.87	0.87	0.87	0.88	0.88
C *n*-butanol:acetic acid:water (60:15:25 v/v) ascending	0.78	0.76	0.76	0.76	0.86	0.86
		0.86	0.86	0.86	0.90	0.90
		0.94			0.94	0.94
D *n*-butanol:acetic acid:water (60:15:25 v/v) descending	0.79	0.75	0.75	0.75	0.85	0.85
		0.84	0.84	0.84	0.89	0.89
		0.95			0.95	0.95
E *n*-butanol:pyridine:water (1:1:1 v/v) descending	0.77	0.75[c]	0.75	0.75	0.83	0.83
		0.83	0.83	0.83	0.86	0.86
		0.87	0.87	0.87	0.90	0.90
		0.96			0.96	0.96

[a] Isolates 1, 2, and 3 were *H. carbonum* race 1; isolates 4 and 5 were race 2. Victoxinine reacted with both bromcresol green and iodoplatinate reagents. Unknown amines reacted with bromcresol green reagent but not with iodoplatinate reagent.

[b] The streak is resolved into three different spots by descending chromatography.

[c] Four different spots were found by using this solvent system.

used by Nishimura et al. (2) and sprayed with 1% w/v iodine in carbon tetrachloride. However, when the descending technique was used, this streak was resolved into at least three discrete spots. Spots with the R_F of victoxinine were not obtained with other solvent systems (Table 1). Furthermore, several color tests indicated that victoxinine was not present, even on paper developed by the technique used by Nishimura et al. Iodoplatinate reagent (8) was the most useful for these color tests; the victoxinine spot on chromatograms gave a strong, instantaneous reaction and a blue-black color, whereas the other substances with similar R_F values did not react. Each of the amines that appear similar to victoxinine gave a blue spot on chromatograms with bromcresol green reagent (8). In solvent system E (Table 1), background color on the paper, treated with bromcresol green reagent, was blue, and the victoxinine spot did not show. Under these conditions, the other amines appeared as green spots.

Victoxinine could also be positively identified by the formation of a crystalline complex with mercuric chloride, which is insoluble in N HCl (4). No other amine found in these culture filtrates has this property. Unfortunately, considerable concentration and preliminary purification was needed in order to detect victoxinine by this test, so it is not useful for survey work.

Although it is clear that none of the 5 *H. carbonum* isolates, examined by Nishimura et al. (2), produces victoxinine, the compounds they judged to be victoxinine have not been isolated or characterized. It is possible that the host-specific toxin or carbtoxinine was responsible for the spots found in strains of race 1 (5). However, when these compounds, which, like victoxinine, react with iodoplatinate, were removed by extraction with chloroform and butanol, a mixture of amines still remained. The composition of this mixture varied among strains and it was not possible, from these experiments, to indicate any substance common to all strains tested.

LITERATURE CITED

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