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Secalonic Acid D: Natural Contaminant of Corn Dust

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The mycotoxin secalonic acid D was detected for the first time as a natural contaminant of corn dust obtained from grain storage elevators. Secalonic acid D amounts ranged from 0.3 to 4.5 ppm (0.3 to 4.5 μg/g), as determined by reversed-phase high-performance liquid chromatography.

The toxic (4, 8), teratogenic (7), and possibly mutagenic (5) ergochrome secalonic acid D (SAD) is a metabolite produced in large amounts by *Penicillium oxalicum* cultured on corn under laboratory conditions (2). Although *P. oxalicum* has been shown to be one of the principal fungal contaminants of corn grown in the midwestern United States (1, 3), no report has demonstrated the presence of SAD in corn. We report here that SAD was present at levels ranging from 0.3 to 4.5 ppm (0.3 to 4.5 μg/g) in 9 of the 12 samples of dust examined.

Samples of settled dust were collected from four grain elevators along the lower Mississippi River near New Orleans. These elevators store grains grown in the midwestern and southern United States and intended for shipment overseas. Settled dust was also collected from a grain elevator in Manhattan, Kans. The dust was stored at −20°C before extraction. Samples (20 g) of corn dust from five elevators and soybean dust from one elevator were each shaken with 100 ml of ethyl acetate for 2 h at room temperature. After filtration, the ethyl acetate was removed, and the oily residue was suspended in 1 ml of chloroform–9 ml of hexane and chilled at 5°C overnight. Similar-sized dust samples not containing SAD were also spiked with 50 and 100 μg of SAD standard (254 to 255°C mp; crystallized from $CHCl_3$ [from *P. oxalicum*]) to ensure adequate recovery under the extraction conditions.

The hexane precipitate was collected by centrifugation and dissolved in 1 ml of acetonitrile-water-tetrahydrofuran-acetic acid (4:3:0.5:0.5 [vol/vol]), the solvent used for reversed-phase high-performance liquid chromatography (HPLC), filtered through a Teflon filter (pore size, 0.45 μm), and analyzed on a 25-cm LiChrosorb RP18 column (Brownlee Laboratories) by the HPLC method of Reddy et al. (6). Yields of SAD were determined by the peak areas (peak retention time, 4.90 min; k′, 2.40) of chromatograms monitored at 340 nm (Fig. 1). Recovery of SAD from spiked samples was greater than 95%. Identical retention times of the peak and the authentic SAD and coelution of the standard and the sample, which were injected simultaneously, were found (Fig. 1). Coelution occurred in the above solvent and in a second solvent (identical to that described above except that the acetonitrile proportion was 5) in which the SAD peak eluted more quickly (retention time with a flow rate of 1.5 ml/min, 3.74 min; k′, 1.74).

The concentrations of SAD in dust obtained from grain elevators ranged from 0.3 to 4.5 ppm (Table 1). SAD was found in 12 of 15 corn dust samples but was not detected in soybean dust. For further confirmation of the presence of SAD in samples positive for SAD, 100 μl of the dust extract was spotted on a Whatman LHP-KF silica plate (10 by 10 cm) for thin-layer chromatography. The plate was developed with benzene-ethyl acetate-formic acid (100:40:10 [vol/vol]). Although a number of contaminating, UV light-absorbing compounds were present, upon spraying with 1% ferric chloride, a red-brown spot characteristic of SAD (9) which had the same R_f as that of authentic SAD and which comigrated with SAD when spotted simultaneously developed. Coelution was also confirmed on silica gel GF254 plates sprayed with 6% tartaric acid and eluted with methyl isobutyl ketone-chloroform (2:8 [vol/vol]; R_f, 0.25).

SAD was also isolated on a preparative scale from 500 g of dust (Manhattan elevator sample 2 containing 0.5 ppm of SAD) by extraction with ethyl acetate. The SAD peak was separated by HPLC, and its UV spectrum was measured. Although the UV spectrum indicated that the sample still contained impurities, a peak at λ_{max} = 335 nm similar to that of authentic SAD (λ_{max} = 338 nm) (6) was found. Unambiguous identification of SAD in this sample was accomplished by mass spectroscopy, using direct inlet introduction of the sample. A direct comparison of

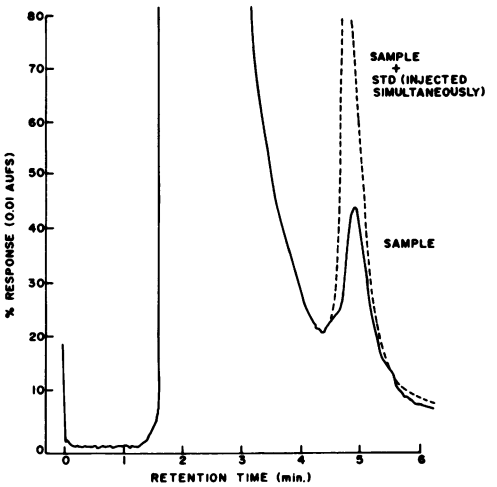

FIG. 1. HPLC chromatogram of a grain dust extract containing SAD (sample from New Orleans elevator [sample b]). For simultaneous injection, 10 μl of the sample was mixed with 10 μl of standard (STD) containing 33 μg of SAD per ml. AUFS, Absorbance units (full scale).

TABLE 1. Yields of SAD from grain dust

Grain elevator location	Grain	Amt of SAD (ppm) in[a]:	
		Sample 1	Sample 2
1. New Orleans	Corn	4.5	2.0
2. New Orleans	Corn	0.9	
3. New Orleans (a)[b]	Corn	0.3	0.4
4. New Orleans (b)[b]	Corn	4.1	1.2
5. New Orleans	Soybean	0[c]	
Manhattan	Corn	3.5	0.5
6. New Orleans	Corn	0	0

[a] The amount of SAD was calculated from the area of the HPLC peak eluting at 3.74 min by the method of Reddy et al. (6). The actual amount recovered from the Manhattan elevator dust (sample no. 2) by preparative thin-layer chromatography and HPLC was 0.027 ppm. Samples 1 and 2 were taken from different locations in the same elevator.

[b] Samples a and b were obtained from the same elevator in November and May, respectively.

[c] The minimum level of SAD detectable by HPLC under the conditions used was 40 ppb (10 ng injected, with detection at 340 nm; 0.01 absorbance units [full scale]).

the 638 molecular ion of the dust extract and the 579 ion of authentic SAD was established. These results demonstrate that SAD is a natural contaminant of corn and is found in detectable amounts in corn dust. In addition, we have cultured *P. oxalicum* from samples of corn dust and therefore presume the mold was present in the corn stored in the elevator.

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