pounds. Since these arise in aqueous media, it is likely that they may result from attack of the S_n1 carbonium ion intermediate on **I** (to form **XII**) or reaction of this intermediate with **XI** (to form **XIII**). Recent studies (Camps et al., 1985) have identified a thermal dimerization product of **I** having properties similar to **XII** in which the two monomers are joined to form a tetrahydrofuran ring and one of the monomeric units bears a hydroxyl group at the 3-position. Although the NMR data for the thermal dimer and **XII** are similar, we found no spectral evidence for the presence of a hydroxyl group in **XII** (Table III), which suggests that these compounds are not the same.

Registry No. I, 62471-06-1; II, 74094-54-5; II-Ac_2, 74094-47-6; III, 74094-53-4; III-Ac_2, 74094-48-7; IV, 95421-28-6; IV-Ac, 95421-29-7; V, 95421-30-0; V-Ac, 95421-31-1; VI, 95464-50-9; VI-Ac_2, 95421-32-2; VII, 95421-33-3; VII-Ac_2, 95421-34-4; VIII, 95421-35-5; VIII-Ac, 95421-36-6; IX, 95421-37-7; IX-Ac, 95421-38-8; X (isomer I), 95421-39-9; X (isomer II), 95529-70-7; X-Ac_2 (isomer I), 95421-40-2; X-Ac_2 (isomer II), 95529-71-8; XI, 95421-41-3; XI-Ac, 95421-42-4; XII, 95421-43-5; XIII, 95421-44-6; XIII-Ac, 95421-45-7; precocene II, 644-06-4; adenine, 73-24-5; morpholine, 110-91-8; thiophenol, 108-98-5; L-cysteine methyl ester, 2485-62-3; methanol, 67-56-1; water, 7732-18-5.

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Volatile Compounds Inhibiting *Aspergillus flavus*

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The volatile compounds, *trans*-2-hexenal, 2,4-hexadienal, furfural, β-ionone, and 1-nonanol, found to occur naturally in corn ears, inhibit the growth of *Aspergillus flavus*. The order of activity for these natural compounds and synthetic analogues was found to be aldehydes > ketones > alcohols. Reduction of active aldehydes and ketones to the corresponding alcohols reduced inhibitory activity. Selected methyl ketones, terpene hydrocarbons, and terpene hydrocarbon oxides were not active.

Moldy food toxicoses affect animal and human health worldwide. Aflatoxin, in particular, has been identified in many foods as a result of fungal infection with certain members of the *Aspergillus flavus* group, notably *A. flavus* Link and *A. parasiticus* Speare.

Species of the *A. flavus* group often infect corn in the hot, and at times, semiarid summers of the southeastern United States. In this ecological niche species of the *Aspergillus flavus* group compete relatively well with other microorganisms and aflatoxin may contaminate food and feed supplies.

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Corn in the field is most susceptible to spore contamination and growth of *Aspergillus flavus* just after pollination when the silks are yellow/brown in color (Payne 1983; Jones et al., 1980). In subsequent storage following harvest the growth of *Aspergillus flavus* and production of aflatoxin may increase.

Since aflatoxin is not manifest during early stages of corn kernel development and silking, when volatile metabolites would be at peak concentration, Wilson et al. (1981) screened volatile compounds reported by Flath et al. (1978), Buttery et al. (1978), and Cantelo and Jacobson (1979) to be present in corn ears for growth suppression of *A. flavus* and *A. parasiticus*. Among the volatile compounds found the conjugated methyl ketone, β-ionone was observed to induce profound effects on the morphology and development of the conidia of *A. flavus* (CP-22). Further, when 100 ppm β-ionone was added to a shake culture of *A. parasiticus* (NRRL 2999) aflatoxin accumu-

Table I. Growth of *A. flavus* CP-22 as a Percent of Control with Various Amounts of Volatile Compounds

active compd	mol of active compd				
	1	2	5	10	10 ($\times 10^{-5}$)[b]
Aldehydes					
trans-2-hexenal[a]	0	0	0	0	4.4
2,4-hexadienal[a]	80	0	0	0	4.6
furfural[a]	88	61	0	0	6.0
trans-cinnamaldehyde	98	90	c	c	5.0
Ketones					
β-ionone[a]	47	23	19	17	2.4
3-penten-2-one	95	71	0	0	5.2
4-methyl-3-penten-2-one	100	92	80	94	4.3
4-(2-furyl)-3-buten-2-one	103	43	c	c	5.5
Alcohols					
trans-2-hexen-1-ol	101	101	97	88	4.2
2,4-hexadien-1-ol	105	100	84	44	4.4
β-ionol	60	51	36	32	2.4
3-penten-2-ol	99	97	92	84	4.9
4-(2-furyl)-3-buten-2-ol	98	95	97	86	5.0
1-nonanol[a]	78	54	28	25	2.8

[a] Present in corn ears. [b] Applied as neat liquid to filter paper. [c] Not tested.

lation was reduced and growth (dry weight) decreased to 64% of the control.

In the present study other volatiles of the corn ear were assayed for inhibition of *A. flavus*. CP-22, a non-aflatoxin producing strain of *A. flavus*, was used in the study to avoid human exposure to the toxins. After the most active volatile inhibitors of CP-22 were determined, synthetic derivatives, which contained the structural features of the active compounds, were made in an attempt to achieve a maximal inhibitory effect on CP-22 with a single compound (see Table I).

EXPERIMENTAL SECTION

Bioassay. The diameter of growth of *A. flavus* (CP-22) on PDA (potato–dextrose agar) surface was measured 4 days after spore innoculation and germination in the presence of the compounds (treatment) and in the absence of compounds (control). The innoculum, CP-22, isolated from Georgia corn, was maintained on PDA slants. The compounds, all liquids at room temperature except 4-(2-furyl)-3-buten-2-one (Leuck and Cejka, 1958), were applied neat to 4.25-cm diameter pieces of filter paper wedged in the flask top and suspended 4–5 cm above but not in contact with the surface of the PDA medium. Each assay for a particular compound was placed in a plastic bag or sealed flask and held at room temperature (22–27 °C).

Control assays containing filter paper with no applied compounds were set up simultaneously along with the treatment assays. At the end of the assay growth was recorded. Eight replicates at each concentration were compared with eight control replicates and analyzed by the F test for significant differences at $P = 0.05$.

Preliminary Screen. Compounds showing little or no effect at 10 μL/dish were not studied further.

Compound Sources. All chemicals were purchased from Aldrich except for β-ionol and 4-(2-furyl)-3-buten-2-ol, which were synthesized by the reduction of the corresponding ketones.

Synthesis of β-Ionol. Lithium aluminum hydride (2.3 g) was dissolved by refluxing in 250 mL of anhydrous ether in a 500-mL three-neck flask equipped with a magnetic stirrer. The β-ionone (11.51 g) dissolved in 150 mL of anhydrous ether was added dropwise from an addition funnel at a rate that maintained a slow reflux. When the addition was complete, the reaction mixture was refluxed for 1.5 h and then cooled in an ice bath. A 20% sodium hydroxide solution was added to destroy hydride and precipitate aluminum salts. The remaining clear, colorless solution was filtered, dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give 11.47 g (98.6%) of β-ionol.

Analysis for purity of the product by capillary GLC on a 25 m × 0.3 mm fused silica column coated with SE-54 and temperature programmed from 60 to 280 °C showed a peak at retention time 14.78 min representing 97.3% of the total peak areas. Retention time of β-ionone was 16.90 min.

The IR spectrum of the product did not show the carbonyl absorptions of β-ionone at 1672 cm^{-1} and 1606 cm^{-1} but instead showed a broad hydroxyl absorption centered about 3337 cm^{-1}. The proton NMR spectrum (60 mHz, reference tetramethylsilane 0.00 ppm) showed the geminal dimethyl (6 protons) singlet at 1.05 ppm, carbinol methyl (3 protons) doublet at 1.32 ppm, olefinic methyl (3 protons) singlet at 1.69 ppm, OH singlet at 2.88 ppm, $CH(CH_3)OH$ single proton multiplet centered at 4.14 ppm, single olefinic proton split doublet (i.e., quartet) centered at 5.48 ppm, a single olefinic proton doublet unsplit centered at 6.18 ppm, and the ring methylene envelope of 6 protons at 1.2 to 2.2 ppm.

Synthesis of 4-(2-Furyl)-3-buten-2-ol. 4-(2-Furyl)-3-buten-2-one (20 g) (Lueck and Cejka, 1958) was mixed with 80 mL of absolute ethanol and 6 g of sodium borohydride in portions with swirling and cooling in a water bath. After addition was complete, the reaction mixture was allowed to stand at room temperature for 72 h, then poured into 500 mL of cold water, and extracted with three 100-mL portions of chloroform. The chloroform extract was dried over sodium sulfate, filtered, and concentrated under vacuum to yield a residue of 16.85 g (83.0%).

Capillary GLC analysis as described above showed 94.4% purity for the ketone starting material (retention time, 7.69 min) and 94.2% for the product alcohol, 4-(2-furyl)-3-buten-2-ol (retention time, 7.12 min).

The IR spectrum of the product alcohol showed the loss of the conjugated carbonyl absorptions at 1613 and 1623 cm^{-1} present in the starting ketone but showed the broad hydroxyl absorption centered at 3367 cm^{-1}.

The proton NMR spectrum (60 mHz, Me_4Si reference 0.00 ppm) of the product alcohol showed a methyl doublet at 1.30 ppm, OH singlet at 3.00 ppm, methinyl proton multiplet centered at 4.43 ppm, olefinic proton complexes (4 protons) at 5.90–6.60, and the single proton at C-4 on the furan ring at 7.30 ppm.

RESULTS AND DISCUSSION

Table I shows compounds which were inhibitory in the bioassay and derivatives for comparison. As a chemical group the aldehydes were the most active inhibitors of growth of *A. flavus*, followed by the ketones and finally the alcohols. Inhibition of growth by the alcohol, *trans*-2-hexenol, was substantially less than the corresponding aldehyde, *trans*-2-hexenal. In every case the reduction of an aldehyde or ketone to the corresponding alcohol reduced activity. However, β-ionol retained much of the activity of the parent compound β-ionone.

The most active synthetic compound, not known to occur in corn, 3-penten-2-one, was reduced in activity by substitution of a second methyl group for hydrogen at the distal end of the double bond as in 4-methyl-3-penten-2-one.

Some compounds tested in the preliminary screen were relatively inactive and were not examined further. The inactive compounds were a series of methyl ketones, 2 butanone through 2-decanone and cyclopropyl methyl

ketone. Other compounds inactive against CP-22 were the terpene hydrocarbons (−)-β-pinene, (+)-α-pinene, *dl*-α-pinene, and the terpene ethers, α-pinene oxide, β-pinene oxide, and cineole. In contrast, α- and β-pinene and cineole have inhibitory activity against higher plants (Muller et al., 1964). The bioassays of these compounds demonstrated that chemical structure is important for activity and that the fungus is not generally sensitive to volatile compounds in the C-4 to C-10 range of molecular weights.

The most active compounds were *trans*-2-hexenal, 2,4-hexadienal, furfural, β-ionone, and 1-nonanol which occur naturally in the corn ear (Flath et al., 1978; Jones et al., 1980; Lueck and Cejka, 1958). β-Ionone also reduced sporulation of *A. flavus* CP-22 but allowed growth with delayed morphological development (Wilson et al., 1981).

The inhibitory action of these volatile, naturally occurring compounds perhaps serves as a barrier to pollination to protect the embryos during the silking stage from intrusion of pollen or spores of other species and thus may enhance the survival rate of the embryos. For instance mycelial growth of *A. flavus* has been found to proceed along the silks (Payne, 1983; Jones et al., 1980). Thus, an additional effect of the volatile inhibitory compounds may be that the young kernels are protected from mycelial growth of invading fungi.

This implies that resistance to *A. flavus* may be bred into corn by selecting for strains that produce higher levels of volatiles. Further, if 2,4-hexadienal could be induced to oxidze to the well-known fungal inhibitor, sorbic acid (2,4-hexadienoic acid), a longer lasting effect of fungal inhibition might be obtained by decreasing the loss of the inhibitors by volatilization. The possibility of further manipulation of the corn plant metabolites with plant growth regulators also exists.

Registry No. *trans*-2-hexenal, 6728-26-3; 2,4-hexadienal, 80466-34-8; furfural, 98-01-1; *trans*-cinnamaldehyde, 14371-10-9; β-ionone, 79-77-6; 3-penten-2-one, 625-33-2; 4-methyl-3-penten-2-one, 141-79-7; 4-(2-furyl)-3-buten-2-one, 623-15-4; *trans*-2-hexen-1-ol, 928-95-0; 2,4-hexadien-1-ol, 111-28-4; β-ionol, 22029-76-1; 3-penten-2-ol, 1569-50-2; 4-(2-furyl)-3-buten-2-ol, 4229-85-0; 1-nonanol, 143-08-8.

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Canavanine to Arginine Ratio in Alfalfa (*Medicago sativa*), Clover (*Trifolium*), and the Jack Bean (*Canavalia ensiformis*)

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The canavanine to arginine ratio (C/A) was determined in six alfalfa varieities (*Medicago sativa*), three clover varieties (*Trifolium*), and the jack bean (*Canavalia ensiformis*). A low-pressure chromatographic procedure was used with a Dowex 50-x-8 column (0.5 × 30 cm) for the separation of the guanidino compounds. Fluoroscence was measured (excitation 307 nm, emission 447 nm) after heating the column eluate with phenanthrenequinone in 2 M NaOH, cooling, and acidifying with 4 M acetic acid. The C/A g kg^{-1} ratios found were for the jack bean 30.9, for the alfalfas Team 11.6, Classic 10.7, Weevlchek 9.78, Saranac A.R. 9.65, Arc 9.55, and Buffalo 8.65. For the clovers the C/A ratios were red 0.824, ladino 0.779, and white 0.600. The canavanine content (g kg^{-1}) of the alfalfas ranged from 8.33 for the least weevil resistant Buffalo to 13.6 for the highly weevil resistant Weevlchek. The other weevil resistant varieties, Saranac A.R. 10.9, Arc 10.8, Team 10.7, and Classic 9.90, were not significantly different from each other.

INTRODUCTION

The distribution of canavanine in the seeds of the leguminosae has been used to classify the various species. (Bell, 1950; Birdsong, et al., 1960; Bell and Tirimann, 1965; Turner and Harborne, 1967; Tschiersch, 1961; Mannhalter and Michl, 1974). Canavanine is toxic to animals, insects, and other plant pests (Tschiersch, 1962; Isogai et al., 1973). Its production appears to be a defense mechanism (Rosenthal, 1977). Alfalfas (*Medicago sativa*) contain substantial quantities of canavanine in their seeds, and it has been suggested that the difference in the resistance of various alfalfa cultivars to the alfalfa weevil (*Hypera postica*) is due, at least partly, to their difference in canavanine content (Natelson and Bratton, 1984; Natelson, 1985).

The toxicity of canavanine is due, largely, to its similarity in structure to that of arginine (Hegdekar, 1970; Vanderzant and Chremos, 1971; Crine and Lemieux, 1982). Canavanine contains an oxygen linked to the guanidino group instead of a carbon. In Figure 1 the structures of canavanine and arginine are compared.

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