A Field Indicator in Plants Associated with Ergot-Type Toxicities in Cattle

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Ergosta-4,6,8(14),22-tetraen-3-one (I) and ergosta-4,6,8(14)-trien-3-one (II) were isolated and identified from *Balansia epichloë* (Weese). This fungus was found parasitizing a fescue (*Festuca arundinacea*) pasture on which cattle showed the clinical signs of fescue toxicity. Compound I also was demonstrated in toxic *Cynodon dactylon* (L.) Pers. (Bermuda grass) and in isolates of *Claviceps* sp. A combined thin-layer gas chromatographic method of analyses of compounds I and II in forage samples is described. Possible use of the ergostaenes as field indicators of ergot-type toxicities associated with livestock and pasture grass is suggested. Compound II has not been previously reported.

Taxonomic relationships between the genera *Balansia* and *Claviceps* have led some investigators (Diehl, 1950; Groger, 1972) to suggest that the Balansiae, if ingested, might also be toxic to man and animals. Nobindro (1934) reported that cattle grazing a pasture grass (*Andropogon aciculatus*) parasitized by a *Balansia* sp. showed symptoms indistinguishable from those of ergot poisoning.

In a screening program (Porter et al., 1973, 1974) designed to discover field indicators of toxic fungi associated with ergot-type syndromes in cattle, *Sporobolus poiretii* (Roem. and Schult.) Hitchc. (smut grass) was found to be heavily parasitized by *Balansia epichloë* (Weese). This fungus also was isolated from *Andropogon* sp., *Eragrostis* sp., and *Festuca* sp. (Bacon et al., 1975). These grasses were growing in a fescue pasture, *Festuca arundinacea* Schreb. [=*F. elatior* var. *arundinacea* (Schreb.) Celak] and cattle grazing this pasture showed a gangrenous ergot-type syndrome called "fescue foot" (Yates, et al., 1969). In addition to the obvious clinical signs of dry gangrene of the rear feet and tail, the fescue toxicity syndrome includes: long, dull hair coat, general poor performance, and elevated temperature and respiration rate. Often, affected cattle stand for long periods in ponds or streams; this may be the only indication of illness (Boman et al., 1973).

Bermuda grass tremors (Porter et al., 1973, 1974) is a convulsive type of ergotism in which symptoms range from a slight twitching or palsy of the muscles in the neck and flank region to an inability to stand or walk because of posterior paralysis. The disorder is associated with cattle grazing common and coastal Bermuda grass (*Cynodon dactylon*) and has been reported from several southern states. Many toxic fungi have been isolated from samples of toxic grass but tests to induce the disorder in cattle were negative (Porter et al., 1973). The specific etiogenic agent(s) remains unknown.

Clinical symptoms indicated that ergot or the related alkaloids were involved with both fescue foot (gangrenous ergotism) and Bermuda grass tremors (convulsive ergotism). We therefore examined *B. epichloë*, isolates of *Claviceps* from toxic Bermuda grass, and toxic and nontoxic grass samples for possible field indicators of these toxicities.

EXPERIMENTAL SECTION

Thin-layer chromatography (TLC) on silica gel (Figures 1 and 2) of an organic extract of both fungi and all toxic grass samples revealed a fluorescent fraction visible at 366 nm. This fraction was not found in appreciable quantities in nontoxic samples, which suggested that it might be used as a field indicator of toxicity.

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Materials and Methods of Culture. Toxic *Cynodon dactylon* (L.) Pers. was obtained from the following areas in which Bermuda grass tremors had occurred (Porter et al., 1973, 1974): Louisiana, 1971 and 1972; Texas, 1972; Mississippi, 1973, Alabama, 1974. Nontoxic samples were obtained from Louisiana, 1971, and Georgia, 1972.

Claviceps sp., strains 174 and 178, were isolated from toxic *C. dactylon* from Mississippi, 1973, cultured as described (Porter et al., 1974) with the addition of yeast, and extracted with chloroform.

Festuca arundinacea Schreb. [= *F. elatior* var. *arundinacea* (Schreb.) Celak] and *Sporobolus poiretii* (Roem. and Schult.) Hitchc., from pastures in Newton Co., Georgia, were labeled (Table I) toxic Ky-31 (1973), smut grass (1972), and smut grass (1973). Toxic and nontoxic standards of Kenhy and Ky-31 fescue (Table I) from R. C. Buckner, Lexington, Ky., were labeled: toxic: Kenhy, 1973; Ky 31, 1973; nontoxic: Kenhy (Rep I and III), 1974; Ky 31 (Rep I and III), 1974; Kenhy Fd, 1974; Ky 31, 1974. Samples that were designated "toxic" were from pastures in which cattle showed clinical signs of fescue toxicity. Samples that were designated "nontoxic" were from plots in which cattle demonstrated generally good performance.

Balansia epichloë (Weese), strain 200 SF, used in this study was isolated from the ascostromata of the fungi found on the adaxial leaf surface of *Sporobolus poiretii* (Roem. and Schult.) Hitchc. This single ascospore strain was maintained on malt extract agar slants at 4°. The contents of a slant was macerated with 10 ml of sterile distilled water and 1.0 ml was used for inoculation.

Fermentations were conducted in 2800-ml baffeled flasks containing 500 ml of a synthetic medium and capped with stainless closures. The medium consisted of: sucrose, 20.0 g; mannitol, 30.0 g; succinic acid, 5.0 g; KH_2PO_4, 5.0 g; NH_4NO_3, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.4 g; $ZnSO_4 \cdot 7H_2O$, 5.0 mg; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 1.0 mg; $MnSO_4 \cdot H_2O$, 0.05 mg; $CuSO_4$, 0.25 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.05 mg; Thiamin, 0.0005 mg; distilled water, 1000 ml; and pH adjusted to 5.5 with concentrated NH_4OH before sterilization (121° for 15 min). After inoculation the cultures were incubated on a rotary shaker (200 rpm, 2.54-cm circular orbit) at 25–27° for 30 days.

Chromatography. All solvents were analytical reagent and all extracts were concentrated in vacuo at 30° or under a stream of nitrogen at ambient temperature.

Column chromatography was performed in a glass column (30 cm × 2.4 cm, i.d.) with silica gel 60 (Brinkmann) of a particle size 0.063–0.200 mm or alumina CC-10 (Mallinkrodt). Extracts were preabsorbed on 10 g of the column material, dried, slurried in the predeveloping solvent, and placed on the chromatographic column material (90 g) prepared as a slurry in either petroleum ether (silica gel column) or hexane (alumina column). The fluorescent fraction was monitored by ultraviolet (uv) at 366 nm as it was elut-

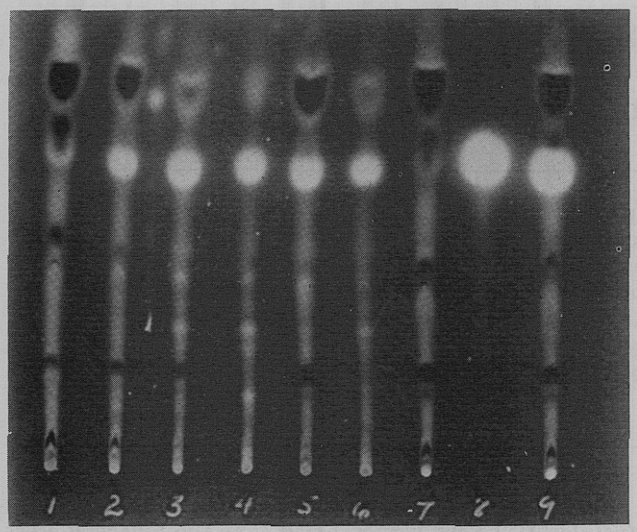


Figure 1. TLC of $CHCl_3$ extract of *Cynodon dactylon* and *Balansia epichloë* on silica gel GF 254; solvent system, $CHCl_3$–$(CH_3)_2CO$ (93:7, v/v); (1) nontoxic Louisiana, 1971; (2) toxic Louisiana, 1971; (3) toxic Mississippi, 1972; (4) toxic Texas, 1972; (5) toxic Louisiana, 1973; (6) toxic Alabama, 1974; (7) nontoxic Georgia, 1972; (8) *B. epichloë* isolate from *Sporobolus poiretii;* (9) *B. epichloë* isolate added to nontoxic Georgia, 1972.

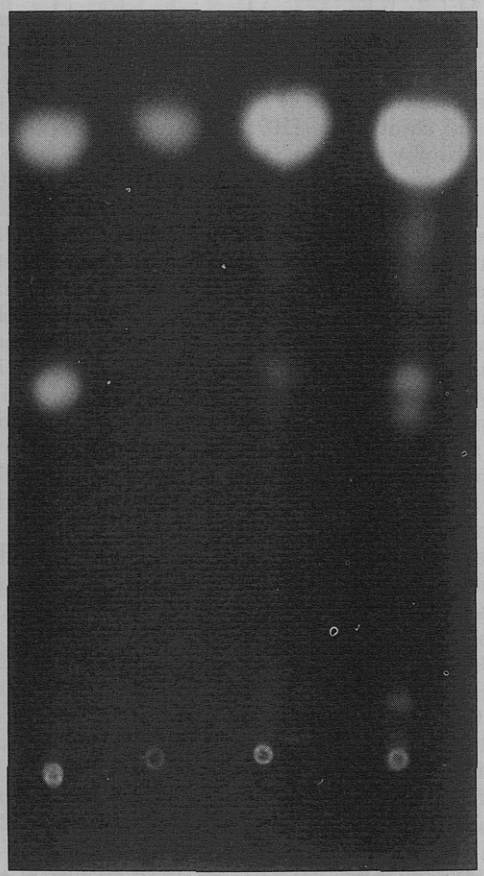


Figure 2. TLC of isolated fluorescent fraction on silica gel GF 254; solvent system, $CHCl_3$–$(CH_3)_2CO$ (93:7, v/v); (1) *Claviceps* 174 plus *yeast;* (2) *Claviceps* 178 plus *yeast;* (3) *Claviceps* 174; (4) toxic *Cynodon dactylon,* Louisiana, 1971.

ed from the chromatographic columns. TLC followed procedures of Porter et al. (1974) and silica gel GF 254 (Brinkmann) of 0.25 or 0.75 mm thickness was used. Developing systems (v/v) were (A) chloroform–acetone (93:7) and (B) chloroform–methanol (199:1). A Perkin-Elmer Model 900 gas chromatograph equipped with a flame ionization detector was used for gas–liquid chromatography (GLC). The stainless steel columns were: (1) 6 ft × 0.25 in. o.d., packed with 3% OV 1 on 100–120 mesh Gas-Chrom Q; (2) 5 ft × ⅛ in. o.d., packed with 3% OV 17 on 100–120 mesh Gas-Chrom Q; (3) 6 ft × ⅛ in. o.d., packed with 3% XE 60 on 100–120 mesh Gas-Chrom Q. Nitrogen was the carrier gas with a flow rate of 40 ml/min. Column temperature was isothermal at 250° and the injector and manifold temperatures were 285°. In addition, column 1 was programmed from 200 to 250° at 8°/min with a 24-min delay before program start. Compounds were trapped in capillary tubes coated with silica gel and the fluorescent material common to all samples was observed visually (at 366 nm) as it emerged from the exit port.

Table I. TLC Results of $CHCl_3$ Extracts of Ky 31 Fescue, Kenhy, and Smut Grass on Silica Gel GF 254; Solvent System $CHCl_3$–$(CH_3)_2CO$ (93:7, v/v)

No.[a]	Sample var. (yr.)	Toxic	Non-toxic	Fluorescent indicator
1.	Ky 31 (1973)	+	−	+
2.	Smut grass (1972)	+	−	+
3.	Smut grass (1973)	+	−	+
4.	Kenhy (1973)	+	−	+
5.	Ky 31 (1973)	+	−	+
6.	Kenhy, Rep I (1974)	−	+	−
7.	Kenhy, Rep III (1974)	−	+	−
8.	Ky 31, Rep I (1974)	−	+	−
9.	Ky 31, Rep III (1974)	−	+	−
10.	Kenhy Fd (1974)	−	+	−
11.	Ky 31 (1974)	−	+	−

[a] Samples 1–3, Newton Co., Ga.; samples 4–11, Lexington, Ky.

Analytical Methods. Molecular weight (M) and compound purity were determined on a DuPont 21492 mass spectrometer. Peak matching techniques were used with perfluorokerosene as a standard. Slit width corresponded to a nominal resolution of 20,000. Samples were introduced via a direct insertion probe with an electron bombardment of 70 eV. Infrared (ir) spectra were obtained on the Perkin-Elmer Model 457A grating infrared spectrophotometer by the micro-KBr pellet technique. Ultraviolet (uv) spectra were measured in ethanol (95%) with the Cary recording spectrophotometer Model 15.

Sample Preparation and Isolation. *Procedures: Forage Samples.* Air dried plant material was ground in a Wiley Mill to pass a 4-mm screen. Samples (2 g) were extracted with chloroform for about 4 hr in a Goldfisch apparatus and the extracts were concentrated to 2 ml. Each sample (20 μl) was spotted on a 20 × 20 cm glass plate coated with silica gel and developed in system A (Figure 1). The fluorescent spots analogous to each sample (R_f 0.66, Figure 1) were scraped from the silica gel, eluted with chloroform–methanol (1:1, v/v), and analyzed by GLC on column 1 (programmed as described). The fluorescent compound present in all fractions had a retention time (t_R) of 57.5 min (programmed 200–250°) and a t_R of 26.5 min at 250° isothermal. Rechromatography on silica gel followed by GLC as described did not change R_f or t_R. A larger scale extraction of toxic *C. dactylon* from Louisiana, 1971, was made in a soxhlet for 24 hr. The concentrated extract was chromatographed on alumina, with increasing percentages of chloroform in hexane. The eluent containing the fluorescent compound was subjected to preparative TLC in solvent system B (R_f 0.60) followed by GLC on column 1 (250° isothermal). Infrared spectra of the pure product and of synthetic compound I are compared in Figure 3.

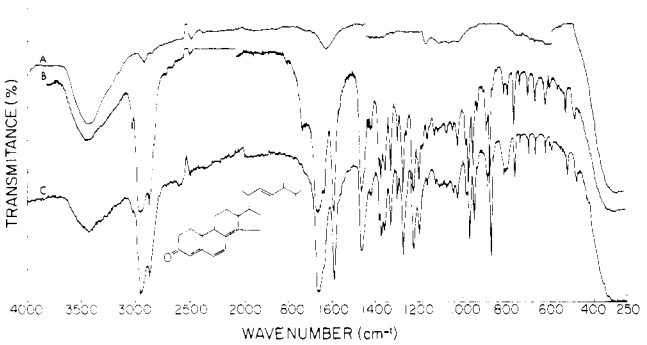


Figure 3. Infrared spectra (KBr) of ergosta-4,6,8(14),22-tetraen-3-one (I): (A) blank KBR, trace water, OH 3450 cm^{-1}; (B) natural product from toxic *Cynodon dactylon*, Louisiana, 1971; (C) synthetic compound I.

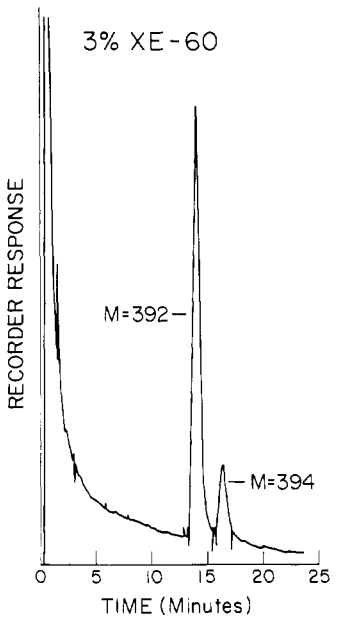


Figure 4. GLC of fluorescent fraction isolated from *Balansia epichloë;* 6 ft × ⅛ in. o.d., packed with 3% XE 60 on 100–120 mesh Gas-Chrom Q; N_2 flow, 40 ml/min; column temperature, 250° isothermal; injector and manifold temperature, 285°.

The extracts from *Claviceps* sp. 174 and 178 were treated as described for the forage samples (Figure 2).

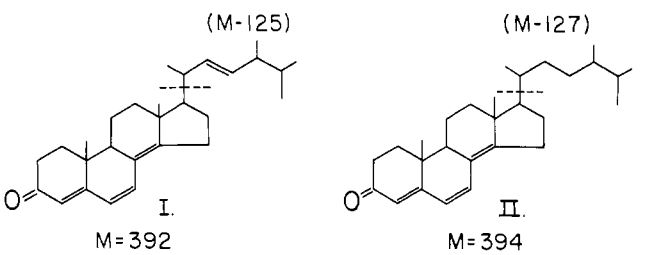
Balansia epichloë. The culture medium (final pH 4.8), prepared as described, was filtered through cheesecloth and the filtrate centrifuged (7500 rpm, 15 min). The medium was decanted and the combined mycelial residues homogenized (Sorvall-Omnimixer) for 5 min in chloroform and filtered by suction, and the extraction repeated (3 × 100 ml). The chloroform extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to about 5 ml. Column chromatography of this material on silica gel first with 350 ml of petroleum ether–chloroform (50:50, v/v) and then with two 250-ml portions of chloroform afforded the fluorescent fraction in the last 250-ml chloroform eluate. The solvent was removed leaving a yellow amorphous residue. This residue was dissolved in 5 ml of chloroform and subjected to preparative TLC in solvent system B. Under uv at 366 nm, three fluorescent bands were observed, so the plates were divided into the following fractions: (a) R_f 0.60; (b) R_f 0.27; (c) R_f 0.20. After extraction from the silica gel, each concentrated eluate was chromatographed in system A. Fraction a remained homogeneous (R_f 0.66) and coincided with the fluorescent fraction isolated from the grass and from *Claviceps* (Figures 1 and 2). Fractions b and c apparently were mixtures of breakdown products (b, R_f 0.58, 0.52; c, R_f 0.60, 0.50, 0.40, 0.33) and were stored at 0° under nitrogen for further investigation.

Table II. High-Resolution Measurements of Steroids Isolated from *Balansia epichloë*

Ion composition	Obsd	Calcd
(I) $C_{28}H_{40}O$	392.3021	392.3079
$C_{19}H_{23}O$ (M − 125)	267.1724 (50%)	267.1749
(II) $C_{28}H_{42}O$	394.3223	394.3235
$C_{19}H_{23}O$ (M − 127)	267.1752 (50%)	267.1749

Identification of the Fluorescent Indicator. The fluorescent fraction a isolated from *B. epichloë*, when subjected to GLC on column 2, separated into two compounds with t_R of 29.5 min (I) and 33.5 min (II). Separation was improved and retention times decreased by using column 3 (Figure 4); t_R = 14 min (I); t_R = 16 min (II). On column 3, the t_R relative to testosterone (Pierce Chemical Co.) were 6.5 min (I) and 8.5 min (II).

Molecular weights (M) were established (Table II) as M = 392 (I) and M = 394 (II). Low-resolution measurements showed that the compounds were uncontaminated. Compound I showed M − 125 = 267 (50%) and compound II showed M − 127 = 267 (50%). The loss of 125 for I and 127 for II both giving 267 (50%) suggested similarities in the fragmentation to the parent ion. High-resolution measurements (Table II) established an empirical formula of $C_{28}H_{40}O$ for M = 392 and $C_{28}H_{42}O$ for M = 394. The uv λ_{max}(EtOH) 348 and the ir spectrum (KBr) V_{max} of 1660, 1640, 1587, 873, and 760 cm^{-1} defined the heteroannular trien-3-one chromophore (Barton and Brunn, 1951; Dorfman, 1953) of compound I. The V_{max} at 967 cm^{-1} supported the trans unsaturation at C-22,23 (Fieser and Fieser, 1959). Synthesis of ergosta-4,6,8(14),22-tetraen-3-one (I) from ergosterol (Aldrich Chemical Co.) as described by Elks (1954), with minor modifications in the procedure, showed that the synthetic and natural products were identical in all respects. The synthetic compound was isolated by chromatography as described for the natural product from *B. epichloë*. Recrystallization from methanol afforded the pure compound: mp 113.0–113.5°; ir (KBr) 3030, 2950, 2870, 1660, 1639, 1588, 1460, 1381, 1370, 1355, 1270, 1220, 1195, 965, 945, 872, and 760 cm^{-1}; uv λ_{max}(EtOH) 348 (ε 27,450), 282 (ε 7450), 240 (ε 5090). These values agree with those reported (Barton and Brunn, 1951; Cooks et al., 1970; Elks, 1954; Endo et al., 1970; Morimoto et al., 1967; Schulte et al., 1968; White and Taylor, 1970; White et al., 1973).



I. Ergosta-4,6,8(14),22-tetraen-3-one

II. Ergosta-4,6,8(14)-trien-3-one

Compound II could be isolated in quantities sufficient for uv, micro ir, and mass spectroscopic analyses. The M = 394 with M − 127 = 267 (50%) suggested the same nucleus as I with the double bond reduced at C-22,23. The heteroannular trien-3-one chromophore was again established by its characteristic λ_{max}(EtOH) 348 and ir spectrum V_{max} 1655, 1640, 1585, 870, and 760 cm^{-1}. Absence of V_{max} at

970 cm^{-1} further supported the C-22,23 saturation, and the geminate dimethyl absorptions at 1375 and 1355 cm^{-1} defined the isopropyl moiety terminating the side chain analogous to ergostane (Weisberger, 1956). Compound II has not been previously reported.

The fluorescent compound isolated from toxic *C. dactylon* was identical with the synthetic compound (Figure 3) and with compound I isolated from *B. epichloë*. Compound I was demonstrated in *Claviceps* 174 and 178 by TLC (Figure 2) and corresponding t_R on column 3.

Toxicity Test. Compound I was tested for toxicity to chicken embryos according to Verrett et al. (1964) with chloroform as a solvent. The following amounts of the synthesized product (Elks, 1954) were used: 10, 25, 50, and 100 μg/egg.

RESULTS AND DISCUSSION

Ergosta-4,6,8(14),22-tetraen-3-one (I) was isolated from samples of toxic *C. dactylon* from Louisiana, Texas, Mississippi, and Alabama (Figures 1 and 3). Repeated outbreaks of Bermuda grass tremors have occurred over the past decade (Porter et al., 1973) in these areas. TLC studies indicate that I is produced by *Claviceps* sp. strains 174 and 178 (Figure 2) isolated from the Mississippi samples. Failure to previously associate *Claviceps* with all samples of toxic hay (Porter et al., 1974) was attributed to the instability of the ergot alkaloids and the time and conditions of sample storage. Isolation and identification of I from all toxic samples of Bermuda grass and from *Claviceps* suggest that this compound might be used as a field indicator of toxic Bermuda grass. Isolation of I from *B. epichloë*, a systemic endophyte found in many grasses in a toxic fescue pasture, also indicates that I could be used as a field indicator of fungal infection of grasses. This is supported by the suggestions of Cooks et al. (1970) that this compound may be utilized as an early indicator of fungal damaged cereals under adverse storage conditions.

The TLC analyses of fescue (Table I) demonstrated the presence of the fluorescent indicator in all toxic samples. The toxicities of *C. dactylon* and *F. arundinacea* probably reflect the level of fungal infestation and concentration of toxic agent(s); thus, sampling time and methodology used to determine the field indicator(s) would be critical. The variability of toxicity within the same pasture and even within a given area coincides with the variability of infected animals within a herd (Porter et al., 1973, 1974). Coincident with this, the fluorescent compound (I) varied among samples randomly selected from bales in lots of hay known to be infected. Also variability was demonstrated within the same bale of toxic hay. This variability supports prior observations (Porter et al., 1973, 1974) that some samples even from the same location are more toxic than others. We are investigating the effects of time on concentration of the ergostaenes from fungi and grasses.

The presence of *B. epichloë* in grasses from toxic fescue pastures and its systematic relationship to the genus *Claviceps* suggested that *B. epichloë* might produce indole or ergot-type alkaloids and thus should be studied in relation to the etiology of fescue foot. Preliminary examination indicated that chloroform extracts of *B. epichloë* were toxic to chick embryos (Bacon et al., 1975) and also that this fungus is capable of producing indole alkaloids in submerged cultures. Results from the chick embryo test indicated that compound I in amounts ranging from 25 to 100 μg/egg was nontoxic. Its toxicity or physiological effects on mammals (ruminants and nonruminants) are unknown.

A species of *Balansia* was isolated from one toxic *C. dactylon* sample (its presence in earlier samples was not looked for) and we are studying this fungus for its ability to produce compound I. The possibility that *Balansia* is involved in both fescue foot and Bermuda grass tremors is being investigated. The endophytic habit and ephemeral fructification of *B. epichloë* (Diehl, 1950) are also being considered with regard to both syndromes.

Compound I has been isolated from *Candida utilis* (Morimoto et al., 1967), *Formes officinalis* (Schulte et al., 1968), *Lampteromyces japonicus* ((Endo et al., 1970), and *Penicillium rubrum* (White and Taylor, 1970; White et al., 1973). It also has been isolated from wheat flour that was infested with several species of *Aspergillus* (Cooks et al., 1970). However, they did not demonstrate that the *Aspergillus* produced the compound and the wheat flour might also have been infected by *Claviceps*. The isolation of compound I from several genera of fungi precludes any utilization of its fluoresence as a means of systematic fungal relationships as previously suggested (Cooks et al., 1970). The ability of other fungi isolated from toxic grasses to produce the ergostaenes is being studied.

The appearance of compound II and the earlier described (Cooks et al., 1970) C-24 ethyl analog of I in the same extract should be considered when using ϵ (λ_{max} (EtOH) 348) as the only means for the specific quantitation of I. Elks (1954), Morimoto et al. (1967), and Cooks et al. (1970) report an ir band at 1550 or 1565 cm^{-1}. This band is absent in the synthetic and natural products we have described (Figure 3). Schulte et al. (1968) did not report this band in their natural product, thus indicating that the band reported earlier was a contaminant. The slightly higher ϵ 27,450 obtained for the synthetic compound also indicates a higher degree of purity.

Compound II was not detected in either *Claviceps* or toxic *C. dactylon*. This may be due to the variation in the initial procedures and limited quantity of samples. Its initial existence in trace quantities in these samples, however, is not precluded.

For screening purposes we suggest using TLC of a chloroform extract of plant material in solvent systems A and B as described. Ultraviolet analyses of the fluorescent fraction eluted from the silica gel should demonstrate absorption at λ_{max}(EtOH) 348, indicative of the heteroannular trien-3-one chromophore. Compound I identification should coincide with retention time relative to testosterone on column 3 as described.

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Aflatoxin Contamination of Corn in the Field

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Aflatoxin has been found in corn samples at all stages of development and maturity from the late milk stage until harvest. Insect damage was observed in 90% of the samples that showed bright greenish-yellow (BGY) fluorescence normally associated with the presence of aflatoxin in cotton seed and corn. A program of spraying insecticide, as recommended to sweet corn growers, reduced the incidence of worm damage an average of 97.7% and other insect damage an average of 92.1%. Stressed growing conditions, such as dense population of plants or reduced fertilization, appear to have a positive influence on the incidence of contamination by aflatoxin. The highest incidence of aflatoxin was found in the warmer, more humid growing regions of the country.

The subject of aflatoxin has become well known in the last 10 to 15 years. Studies conducted with animals have shown it to be an extremely toxic compound; however, the extent of toxicity to man is not as well understood. The major producers of the toxin appear to be the molds *Aspergillus flavus* and *Aspergillus parasiticus*. Diener and Davis (1969) report the presence of aflatoxin in bermuda grass, hay, soybean meal, oats, cassava, corn, peas, rice, soybeans, wheat, cow peas, sesame, sorghum, sweet potatoes, etc.

Christensen and Kaufmann (1968), Golumbic and Kulik (1969), and others have published studies relating aflatoxin to grain stored at high moisture and temperatures. This paper reports on studies conducted over a 3-year period, by the Quaker Oats Company, associating aflatoxin with corn before and during harvesting.

1971 STUDY

The first field survey was a result of low levels of aflatoxin appearing in by-product (feed) material from a corn mill in the spring of 1971. A careful survey and sampling program for in-plant contamination produced negative results from corn storage to finished products. Since no evidence of mold growth conditions was found in the plant, attention was directed to incoming corn. Was the corn contaminated at time of purchase or was it occurring during storage? A comprehensive field survey and sampling program was developed for the 1971 crop, covering essentially all the corn producing areas of the United States. Sampling started 6 weeks prior to harvest and continued through harvest and subsequent handling as long as identity could be maintained.

Arrangements were made with some of the farmers to let a small area of corn in the field stand 3 months beyond harvest. Periodic samples were taken during this time to determine if aflatoxin developed in corn after maturity in the field.

Sample Examination Procedure. The sample, either ear or shelled corn, was first subjected to inspection under "black light" (long-wave ultraviolet, 365 nm), for the characteristic bright greenish-yellow (BGY) fluorescence normally associated with the presence of aflatoxin in cotton fibers as reported by Marsh et al. (1969). Evidence indicates that the fluorescing substance in cotton fibers is formed by a heat-liable enzyme in the living plant that oxidizes kojic acid produced concurrently with aflatoxin by *A. flavus*. This method was adapted to corn and reported by Shotwell et al. (1972).

Kernels of corn and material that fluoresced under the black light were analyzed by the thin-layer chromatography (TLC) method as shown in Official Methods of AOAC (1970), to confirm the presence of aflatoxin. The duckling feed test, as reported by Sargeant et al. (1961), was also used in some of the early samples for confirmation of aflatoxin.

Results. Aflatoxin was found in the first field sampling when the corn was in the late milk stage. There appeared to be no additional aflatoxin development in the standing corn remaining in the field after harvest. Contamination was found in most growing areas but the highest incidence of aflatoxin was in the warmer, more humid growing regions of the country and followed the same geographical pattern as found in cottonseed and reported by Marsh and Taylor (1958).

Table I shows results of some field samples that were sieved over $^{18}/_{64}$ and $^{16}/_{64}$ in. round hole perforated (RHP) screens; the sized fractions were picked under ultraviolet light to remove the fluorescing material and analyzed by TLC method to confirm aflatoxin.

Table II shows the analytical results of a field sample where the corn was picked for fluorescing material before analyzing for aflatoxin, and an ear with several fluorescing kernels and the first and second rows of kernels surrounding the fluorescing kernels.

1972 STUDY

The objective of the 1972 program was to determine how and when the aflatoxin producing mold spores invaded the corn kernels. This study was confined to two geographical areas where field contamination had been found the previous year and the Quaker Oats Company Research Farm

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