

The aflatoxin Q₁ produced in the rat liver system was identified by co-chromatography with the major aflatoxin metabolite of the monkey liver system using various tlc systems. Approximately one-third of the incubated aflatoxin B₁ was metabolized to aflatoxin Q₁ by the monkey liver microsomes. Another one-third of radioactivity was retained in the origin region of the developed tlc plates. None of the five fluorescent metabolites were formed when heat deactivated microsomes were used in control experiments. When 10-ml volumes of the reaction mixture were incubated in 50-ml baffled conical flasks in the *in vitro* metabolic studies (Dalezios *et al.*, 1972), as much as 52% of aflatoxin B₁ was converted to aflatoxin Q₁. The relatively low percentage conversion observed in the preparatory runs was perhaps due to the insufficient oxygen supply in the 100-ml volumes. The significant conversion of aflatoxin B₁ to Q₁ was also observed in similar experiments using microsomal preparations from livers of two other species of monkeys *Macaca irus* and *Saimiri sciureus*. The extent of conversion ranged from 16 to 41% of the added aflatoxin B₁ at a substrate level of 0.2–1 mg of aflatoxin B₁ per gram equivalent of liver.

Compared to monkey liver preparations, rat liver microsomes possessed much lower catalytic activity for conversion of aflatoxin B₁ to Q₁. Only 2–3% of aflatoxin B₁ was biotransformed to Q₁. Unfortunately this low activity makes readily available rat liver microsomes an unsuitable enzyme source for the production of Q₁. Production of aflatoxin Q₁ so far has not been reported but the green fluorescing metabolite of aflatoxin B₁ in rat liver with similar tlc R_f values as recently described by Friedman and Yin (1973) was probably also aflatoxin Q₁.

Approximately 20% of the substrate was converted to non-chloroform-extractable materials. The loss of substrate could have been due to the formation of aflatoxin B₁ hemiacetal, followed by degradation of this compound

in the phosphate-buffered protein solution (Patterson and Roberts, 1970). These materials have not been further characterized as they do not interfere with the production of aflatoxin Q₁.

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Toxic Effects of Oosporein from *Chaetomium trilaterale*

Richard J. Cole,* Jerry W. Kirksey, Horace G. Cutler,¹ and Elmer E. Davis²

A toxin-producing strain of *Chaetomium trilaterale* was isolated from moldy peanuts. A red crystalline metabolite, subsequently purified from the fungal culture broth, had a median oral lethal dose of 6.12 mg/kg in day-old cockerels. The

toxin also produced plant growth inhibiting and phytotoxic effects. The toxin was conclusively identified as the dibenzoquinone, oosporein (3,3',6,6'-tetrahydroxy-5,5'-dimethyl-2,2'-bi-*p*-benzoquinone).

Chaetomium spp. have been isolated from various agricultural commodities including feeds and food products such as peanuts, rice, flour, spaghetti products, and black and red pepper (Christensen *et al.*, 1968; Kurata *et al.*, 1968; Hanlin, 1973). Christensen *et al.* (1968) reported that 83% of the *Chaetomium* spp. isolated from feeds and foods were lethal to rats. In this study the toxigenic factors were not elaborated. In an unrelated study, Christensen *et al.* (1966) showed that toxicity of moldy corn infected with *Chaetomium globosum* was due to chetomin, an

epipolythiadioxopiperazine compound. Brewer *et al.* (1971) reported that *Chaetomium* spp. were implicated in the growth syndrome of sheep in Nova Scotia.

We wish to report the isolation of a toxigenic isolate of *Chaetomium trilaterale* Chivers from moldy peanuts, and the chemical identification of the major toxic metabolite as oosporein.

MATERIALS AND METHODS

Isolation of Toxin. *Chaetomium trilaterale* Chivers (ATCC accession number 24912) was cultured in Fernbach flasks (2.8 l.) containing 200 ml of Difco mycological broth (pH 4.8) supplemented with 2% yeast extract and 15% sucrose. After 14-days growth at 28°, the culture broth was acidified to pH 2.0 and extracted three times with ethyl acetate. Toxicity was associated with a red crystalline material that formed after concentration of the

*National Peanut Research Laboratory, U. S. Department of Agriculture, Dawson, Georgia 31742.

¹U. S. Department of Agriculture, Department of Plant Physiology, Georgia Coastal Plain Experiment Station, Tifton, Ga. 31794.

²American Type Culture Collection, Rockville, Md. 20852.

ethyl acetate solution. The toxic red compound was recrystallized from ethyl acetate solution.

Vertebrate Bioassay. One-day-old DeKalb cockerels (38 g average) were used for vertebrate bioassay. The filtered fungal culture broth was dosed directly to cockerels by crop intubation (1 ml/cockerel). Purified toxin was prepared for dosing by dissolving it in ethyl acetate, adding the desired amount of corn oil to the ethyl acetate solution, and removing the ethyl acetate under vacuum at 70°. These preparations formed what appeared to be a solution or a fine, uniform suspension of the toxin in corn oil. Cockerels were administered 1 ml of these corn oil preparations by crop intubation. Dosage levels used for LD₅₀ determination were 200, 300, and 400 µg/cockerel. Sixty replications of each dosage level were used for LD₅₀ determination.

Plant Bioassay. First internode sections were cut 4 mm long from etiolated 5-day-old *Avena sativa* L., Mo-0205 seedlings, and incubated in buffered solutions, at pH 5.6, containing 2% sucrose (Nitsch and Nitsch, 1956) and toxin. Toxin was dissolved in acetone (Cutler, 1968) and added to the assay solution to produce concentrations of 10⁻³, 10⁻⁴, and 10⁻⁵ M. Internode sections were incubated in these test solutions for 24 hr at 21°, in a roller-tube apparatus, then measured (×3 images produced by a photographic enlarger), and the data were statistically analyzed (Kurtz *et al.*, 1965).

Wheat coleoptile sections, cut 4 mm long from 4-day-old etiolated *Triticum aestivum* L., Wakeland, were assayed in an identical manner with oat first-internode sections (Hancock *et al.*, 1964).

Toxin was tested for effects on intact plants by spraying aqueous solutions at concentrations of 10⁻², 10⁻³, and 10⁻⁴ M plus 0.1% Tween 20 onto greenhouse grown tobacco and bean plants. Single applications of 1 ml of test solution containing 3060, 306, and 30.6 µg of toxin, respectively, were applied to 6-week-old tobacco seedlings (*Nicotiana tabacum* L., Hick's). Each experiment was triplicated, and observations were made at 7 and 14 days after treatment. Seven-day-old bean plants (*Phaseolus vulgaris* L., Black Valentine) were treated with a single application of 1 ml of toxin solution per three plants (three plants per pot) at concentrations of 10⁻², 10⁻³, and 10⁻⁴ M (1020, 102, and 10.2 µg per plant). Each treatment was triplicated and observations were made 7 and 14 days after treatment.

Physical and Chemical Analyses. Ultraviolet (uv) spectra of the toxin were taken with a Beckman Model DB-G recording spectrophotometer in 95% ethanol solution. Infrared (ir) spectra were taken with a Perkin-Elmer Model 257 recording spectrophotometer equipped with a 4× beam condenser. Samples for ir analysis were coated as a thin film onto KBr windows. Low-resolution (LRP) and high-resolution (HRP) mass spectral analyses were made with an A.E.I. Ms-9 mass spectrometer. Samples were introduced into the instrument by the direct probe method, and ionization was effected by electron-impact at 70 eV or by chemical ionization with isobutane as the ionizing reagent. Melting points were determined with a Kofler micromelting point apparatus and were uncorrected.

Nuclear magnetic resonance (nmr) spectra of the toxin and its acetate derivative were obtained with a Jeolco 60-MHz nmr spectrometer. Samples of toxin were analyzed in dimethyl-d₆ sulfoxide solution; the acetate derivative was analyzed in equal concentrations of acetone-d₆ and chloroform-d.

The toxin and its acetate derivative were chromatographed on thin-layer plates coated with silica gel GH-R by using toluene-ethyl acetate-formic acid (5:4:1, v/v/v) as the solvent system.

The tetraacetate derivative was prepared by suspending the toxin in acetic anhydride, adding a few drops of concentrated sulfuric acid, and heating at 70° for 1 hr under

nitrogen. The reaction mixture was poured over cracked ice and partitioned into chloroform. The acetate derivative crystallized from chloroform solution after the extract was reduced in volume under vacuum.

RESULTS AND DISCUSSION

Biological Effects on Cockerels. The oral median lethal dose (LD₅₀) of the purified toxin dosed to day-old cockerels in corn oil suspension was 6.12 mg/kg (245 µg/cockerel). At this dosage level, mortalities began to appear approximately 48 hr after dosing. The minimum lethal dose required to kill 100% of the cockerels (LD₁₀₀) was obtained at a dosage level of approximately 10 mg/kg or 400 µg/cockerel. Increasing the dosage level caused death of chicks in a correspondingly shorter period of time.

Biological Effects on Plants. The toxin produced growth-inhibitory and phytotoxic effects in plants. *Avena* first internode sections were significantly ($P < 0.01$) inhibited at 10⁻³ and 10⁻⁴ M; that is, 90 and 66% respectively, relative to controls. At 10⁻⁵ M, the sections were inhibited 27%. A slightly different response was elicited with wheat coleoptiles in terms of relative inhibition. At 10⁻³ and 10⁻⁴ M they were inhibited ($P < 0.01$) 100 and 44% relative to controls and at 10⁻⁵ M, 14% inhibition occurred.

Observations made 7 days after tobacco plants were treated with 10⁻² M solutions of toxin showed stunting, rosetting, leaf puckering, and some phytotoxicity (Figure 1A). At 10⁻³ M there was less leaf puckering, slight chlorosis, and some stunting (Figure 1B). No visible effects were induced by the 10⁻⁴ M solution relative to the controls (Figure 1C).

In bean plants toxin produced marked effects 7 days after treatment. At 10⁻² M, severe stunting occurred in the internode between the first pair of true leaves and the first trifoliate leaf. There was also some modification in the morphology of the first trifoliate leaf (Figure 2A). At 10⁻³ M there was some stunting of the aforementioned internode, the first trifoliate was greatly modified, and leaf puckering occurred (Figure 2B). No effects were noted at 10⁻⁴ M relative to the controls (Figure 2C). The overall stunting effect produced by the shortened internode is clearly visible in Figure 2D.

Fourteen days after treatment at all concentrations new growth in both tobacco and bean plants appeared normal, but the affected plant parts failed to grow normally. The inhibitory and morphological effects produced by toxin were apparently localized and transient as far as new growth was concerned.

Physical and Chemical Characteristics. *Chaetomium trilaterale* produced 2.5 g of toxin/l. of medium after 14-days growth. Purified toxin had a melting point of 245–250° and appeared as a single red spot on tlc plates at R_f 0.70.

Uv analysis of the toxin showed λ_{max}(MeOH) 208 (ε 33,500) and 291 nm (ε 23,000). The ir spectrum showed major absorptions at 3300 (H-bonded OH), 1625 (chelated quinone), and 1380 cm⁻¹ (CH₃).

HRP mass spectral analyses showed a molecular ion peak (m*) at m/e 306.0368 and a molecular formula of C₁₄H₁₀O₈. LRP mass spectral analysis of the acetate derivative via electron-impact ionization and chemical ionization demonstrated that it was a tetraacetate derivative with a nominal mass of m/e 474 and 475, respectively, and with a probable molecular formula of C₂₂H₁₈O₁₂. Fragment ions appeared at m/e 431, 388, 345, and 302 and demonstrated losses of m* - 4-CH₃C(=O) groups.

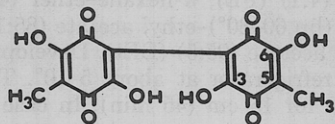
The nmr spectrum of toxin showed only one chemical shift at δ 1.79 (s). The chemical shift was consistent with that expected for the two equivalent 5,5'-CH₃ groups



Figure 1. Effects on tobacco plants 7 days after treatment with toxin at (A) 10^{-2} and (B) 10^{-3} M, and (C) the untreated control.

present in oosporein. The chemical shifts for the 4-OH groups were not visible due to exchange in dimethyl- d_6 sulfoxide. The nmr spectrum of the tetraacetate derivative showed singlets at δ 2.31 ($\text{CH}_3\text{C}(=\text{O})$), 2.19 ($\text{CH}_3\text{C}(=\text{O})$), and 1.99 (CH_3). These data are consistent with those for the expected chemical shifts for the 3,3'-positioned $\text{CH}_3\text{C}(=\text{O})$ groups, the 6,6'-positioned $\text{CH}_3\text{C}(=\text{O})$ groups, and the 5,5'-positioned CH_3 groups in oosporein tetraacetate.

Comparisons of the uv, ir, nmr, mass spectral, and tlc analyses showed that the toxin was identical with oosporein (3,3',6,6'-tetrahydroxy-5,5'-dimethyl-2,2'-bi-*p*-benzoquinone = chaetomidin = isoosporein) (I) (Itahashi *et al.*, 1955; Smith and Thomson, 1960).



I

Although the metabolite oosporein has been reported to be produced by several fungi, including fungi imperfecti (Vining *et al.*, 1962; Itahashi *et al.*, 1955; Smith and Thomson, 1960; Kogl and van Wessum, 1944), ascomycetes (Lloyd *et al.*, 1955), and basidiomycetes (Divekar *et al.*, 1959), this is the first report of its toxicity to animals and plants. The significance of this discovery may be enhanced in view of the widespread occurrence of *Chaetom-*

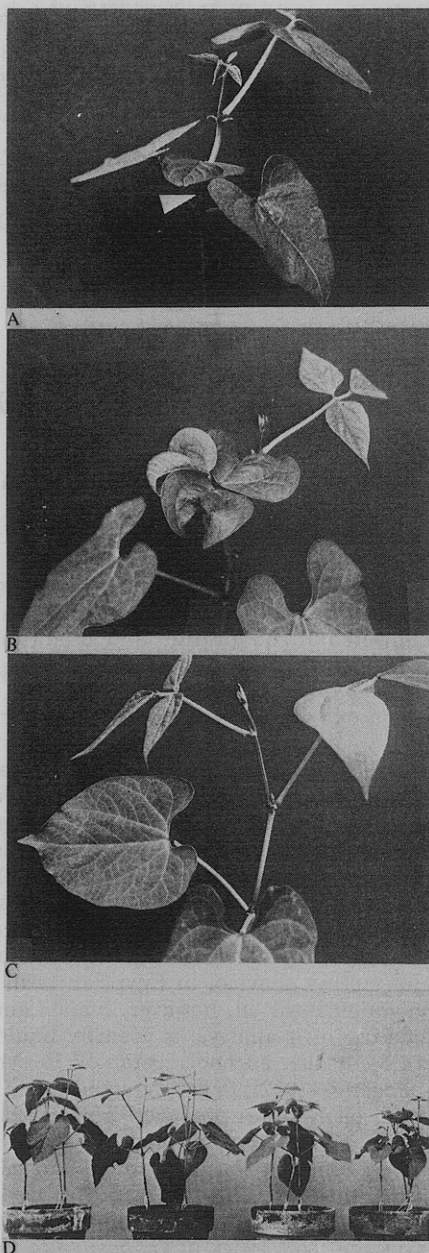


Figure 2. Effects on bean plant internodes 7 days after treatment with toxin at (A) 10^{-2} and (B) 10^{-3} M, and (C) the untreated control; (D) comparison of gross effects on bean plants 7 days after treatment: (left to right) control, 10^{-4} , 10^{-3} , and 10^{-2} M.

ium species and other fungi capable of producing oosporein.

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Oils of *Ocimum basilicum* L. and *Ocimum rubrum* L. Grown in Egypt

Mohamed S. Karawya,* Fayek M. Hashim, and Mohamed S. Hifnawy

Steam-distilled leaf and flower oils of *Ocimum basilicum* L. and *Ocimum rubrum* L. were subjected to qualitative and quantitative study using one- and two-dimensional tlc, glc, and ir

techniques. Many constituents were identified, linalool and methyl chavicol being the most prominent. The physicochemical constants of the investigated oils were also measured.

The oil of *Ocimum basilicum* L. (sweet basil), being employed quite extensively in all kinds of flavors including those for confectionary, baked goods, condimentary products, spiced meats, and sausages, as well as in dental products and certain perfumes (Guenther, 1952), has been the subject of many investigations. Hörhammer *et al.* (1964), using tlc, detected terpineol, linalool, geraniol, citronellol, and possibly nerolidol together with methyl cinnamate and methyl chavicol in Egyptian basil oil. In the case of Bulgarian basil oil, however, Ivanov and Iordanov (1964) made use of ir analysis to identify linalool, methyl chavicol, 1,8-cineole, eugenol, geraniol, *p*-cymene, and myrcene. Pogany (1967) found no qualitative difference between basil oil samples produced from plants grown at different temperatures. Pogany and coworkers (1967) also located benzyl ether in the glc chromatogram of the oil. Nigam and Kameswara (1968) stated that the percentage yield of the oil produced by steam and water distillation of the leaves and soft twigs of *O. basilicum* dried in the shades was 0.8%. They were able to identify methyl cinnamate, methyl chavicol, linalool, cineole, ocimene, borneol, sambulene, and safrole in the oil.

As regards the oil of *O. rubrum*, however, nothing could be traced in the current literature concerning its chemical or physical analysis. The oil was found to occur in a high percentage in the plant and to possess a sweet and fine aroma. On these bases, the oil promises to be of industrial importance.

Accordingly, this work was carried out to present a comparative phytochemical study of the oils produced from *O. basilicum* and *O. rubrum* grown in Egypt.

MATERIALS AND REAGENTS

The following materials and reagents were used during this study: the steam-distilled oils of leaves and flowers of *O. basilicum* L. and *O. rubrum* L. (cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Guiza; the plants were identified through the courtesy of V. Takholm, Faculty of Science, Cairo University, and

collected during the flowering stage in March); reference volatile oil constituents; chromogenic spray, 5% w/v vanillin in sulfuric acid; silica gel for column chromatography and silica gel G Merck for tlc.

APPARATUS

A heated dual flame ionization detector programmed chromatograph, Pye series 104, Model 64, fitted with an RE 511 potentiometric recorder adjusted at 5 mV variable, was used. The apparatus was provided with coiled glass columns, 5 ft long and 4 mm i.d., packed with 10% Reoplex 400 (polypropylene glycol adipate) or 3% SE 30 (methyl silicone polymer) on 85-100 mesh Celite; spectrophotometer Unicam SP 200 was employed for ir study.

PROCEDURES

Determination of Physicochemical Constants. The specific gravity, refractive index, optical rotation, alcohol content, ester content, and solubility in 70% alcohol were carried out according to the Egyptian Pharmacopoea (1963) methods. The results together with the percentage yield of the oils (calculated according to moisture-free basis) are shown in Table I.

Tlc. Ten percent (v/v) of the oils under investigation, as well as reference volatile oil constituents in acetone, were chromatographed on silica gel G plates (20 × 20 cm and 0.3 mm thick) using five developing solvent systems: toluene-ethyl acetate (4:1) (TEt), petroleum ether (bp 60-80°)-ether (4:1) (PE), *n*-hexane-ether (4:1) (HE), petroleum ether (bp 60-80°)-ethyl acetate (85:15) (PEt), and benzene-ethyl acetate (95:5) (BEt). Development was carried out in a refrigerator at about 5-10°. The liquid was allowed to run for 15 cm (45 min). In case of BEt, however, development was operated at room temperature (15-20°) to safeguard against the probable crystallization of benzene during development (20-30 min). The chromatograms were then inspected under uv light and located with the chromogenic spray before and after heating at 105° for 5 min.

Two-Dimensional Tlc. This technique was applied to the oils under investigation using, for the first and second developments, the solvent systems BEt and PEt, respectively.

Column Chromatography. Five-tenths milliliter of

*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.