

Emodin, a Toxic Metabolite of *Aspergillus wentii* Isolated from Weevil-Damaged Chestnuts

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A diarrheagenic toxin from culture extracts of *Aspergillus wentii* Wehmer isolated from weevil-damaged Chinese chestnuts was identified as emodin (2-methyl-4,5,7-trihydroxyanthraquinone). The orange-red, crystalline toxin (mp 255 to 257 C) showed ultraviolet absorption maxima in ethyl alcohol at 223, 250, 267, 290, and 442 nm, and infrared absorption maxima at $3,400\text{ cm}^{-1}$ (OH), 1,635, and $1,625\text{ cm}^{-1}$. Chemical shifts and coupling constants of the proton magnetic resonance spectra of the *A. wentii* toxin and of authentic emodin agreed. Mean lethal dose of emodin orally administered to 1-day-old DeKalb cockerels was 3.7 mg/kg.

Emodin (2-methyl-4,5,7-trihydroxyanthraquinone) is a pigmented fungal metabolite which is regarded as a precursor, or having the type structure, of many of the naturally occurring fungal anthraquinones (3). It was first reported in 1925 as "frangula-emodin," a metabolite of *Dermocybe sanquinens* (Wulf ex Fr.) Fr., by Kögl and Postowsky (5). Emodin has since been identified as one of the pigmented products in culture extracts of *Cladosporium fulvum* Cooke (1), *Penicillium clavariae-formis* (13), *Penicillium brunneum* Udagawa (14), and *Penicillium avellaneum* (Thom et Tureson) (7). Yamazaki (17) identified emodin as one of the toxic metabolites of *Aspergillus ochraceus* Wilhelm.

Aspergillus wentii Wehmer is a cosmopolitan, soil-inhabiting mold found on a variety of organic substrates such as decayed vegetation and moist grains (10). J. M. Wells (Proc. Am. Phytopathol. Soc. 1:103, 1974) frequently isolated *A. wentii* from weevil-damaged Chinese chestnuts and found a majority of the isolates to be toxic to 1-day-old Dekalb cockerels. Rabie et al. (9) first reported *A. wentii* as being toxic to Pekin ducklings, New Hampshire chicks, rabbits, and sheep. Semeniuk et al. (12) found *A. wentii* to be highly toxic to weanling Swiss white mice, but not to Dekalb 131 cockerels.

A. wentii has been reported to produce aflatoxins (6), but confirmatory studies have been few. Schroeder and Verrett (11) found aflatoxins in extracts of *A. wentii* isolated from field corn, but in low and variable amounts. The tests by Rabie et al. (9) for aflatoxin production by *A. wentii* proved negative. The toxicity of *A. wentii* isolated from groundnuts has been at-

tributed to unidentified metabolites other than aflatoxin (8, 9). Wu et al. (16) partly characterized the principle toxin from isolates of *A. wentii* from country-cured hams. The toxin, reported to form orange-red, needle-shaped crystals, was chloroform soluble, with ultraviolet (UV) and visible absorption maxima in chloroform at 270, 295, and 452 nm. However, the *A. wentii* toxin was not fully characterized.

This paper reports the identity and properties of a toxin obtained from a strain of *A. wentii* isolated from weevil-infected Chinese chestnuts in Georgia.

MATERIALS AND METHODS

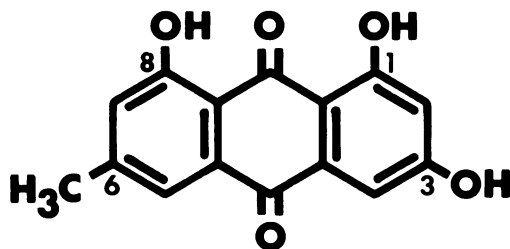
Production and isolation of toxin. *A. wentii* Wehmer was isolated from Chinese chestnuts damaged by the chestnut weevil (*Curculio sayi* Gyllenhal). The organism was cultured in Fernback flasks (2.8 liter) containing 100 g of shredded wheat and 200 ml of mycological broth (pH 4.8; Difco) supplemented with 1.6% yeast extract. After 3 weeks of growth at 28 C, fungal cultures were extracted three times with chloroform in a Waring blender, and the extracts were vacuum filtered through anhydrous sodium sulfate. The filtrate was concentrated and chromatographed on a silica gel column (0.05- to 0.2-mm mesh) by gradient elution from toluene to ethyl acetate. Presence of the toxin was monitored by dosing 1-day-old Dekalb cockerels orally via crop intubation (4) and by silica gel (Mallinckrodt Silicar TLC-7GF) thin-layer chromatography. The developing solvents were toluene-ethyl acetate-formic acid (5:4:1, vol/vol/vol) and chloroform-acetone (93:7, vol/vol). The purified toxin was crystallized from ethanol solution.

Physical and chemical analyses. UV spectra of the toxin in 95% ethanol were determined with a

Beckman model DB-G recording spectrophotometer. Infrared spectra of samples coated as thin films onto KBr windows were recorded by a Perkin-Elmer model 257 recording spectrophotometer equipped with a 4× beam condenser. Low-resolution and high-resolution 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 ionized by electron impact at 70 eV. Melting points were determined with a Kofler micromelting point apparatus and were uncorrected. The proton magnetic resonance spectra of the toxin and of emodin in dimethyl-*d*₆-sulfoxide were obtained with a Jeolco 60 MHz spectrometer.

RESULTS

Chemical and physical properties. The toxic effects of *A. wentii* were due to an orange-red crystalline compound (mp 255 to 257 C) that chromatographed at *R*_f 0.45 (chloroform-acetone) and 0.80 (toluene-ethyl acetate-formic acid) on silica gel layers. UV absorptions were observed at λ ethyl alcohol maxima 223, 250, 267, 290, and 442 nm. Major infrared absorptions were at 3,400 cm⁻¹ (OH), 1,635, and 1,625 cm⁻¹ (quinone carbonyl). High-resolution analysis showed a molecular ion peak, C₁₅H₁₀O₆, at electronic mass 270.0527. These data suggested that the *A. wentii* toxin was identical to emodin (Fig. 1).



EMODIN

FIG. 1. Chemical structure of emodin (2-methyl-4,5,7-trihydroxyanthraquinone).

Proton magnetic resonance spectra data of emodin and of the *A. wentii* toxin can be further compared (Table 1). The chemical shifts and coupling constants of the pair of nonequivalent, meta protons and the methyl protons were in close agreement. The OH protons were not observed, presumably because of exchange.

Biological properties. Crude extracts of emodin administered orally caused severe diarrhea to 1-day-old cockerels. Clinical symptoms included loss of appetite, accumulation of fecal material with acute epidermal irritation around the cloaca, general debilitation, and mortality within 5 days of ingestion.

A purified preparation of emodin was moderately toxic, with a mean lethal dose of 3.7 mg/kg (Table 2). Mortality was 83% at 12.2 and at 37.0 mg/kg. Diarrheagenicity was light in occasional survivors.

DISCUSSION

Comparison of our data with those reported in the literature strongly suggests that the *A. wentii* toxin is emodin. All 5 UV-absorption maxima of the toxin corresponded to those of emodin—222, 252, 265, 289, and 437 nm (15). Agosti et al. (1) reported λ ethyl alcohol maxima at 220, 252, 267, 288, and 435 nm for emodin produced by *Cladosporium fulvum*. Furthermore, the diarrheagenic nature of the *A. wentii* toxin relates to the medicinal use of emodin as a laxative (15).

The toxicity of emodin to 1-day-old cockerels was maximal at 37.0 and 12.2 mg/kg. Emodin partially crystallized from solution at higher concentrations. Diarrheagenicity was acute with crude extracts of emodin and was moderate with purified preparations. The possibility exists of other compounds in the crude extracts enhancing the diarrheagenic activity of emodin.

We propose that the toxin from *A. wentii* partially characterized by Wu et al. (16) was a substituted anthraquinone such as emodin, chrysophanol (2-methyl-4,5-dihydroxyl), or *w*-

TABLE 1. Comparison of proton magnetic resonance spectra of *A. wentii* toxin and of authentic emodin in dimethyl-*d*₆-sulfoxide solution

<i>A. wentii</i> toxin			Emodin		
Chemical shift (δ) ^a	No. of protons	Assignment	Chemical shift (δ)	No. of protons	Assignment
2.34 (s)	3	Ar-CH ₃ ; position 6	2.26 (s)	3	Ar-CH ₃ ; position 6
6.43 (d) (J:2.0 Hz)	1	Ar-H; position 7	6.49 (d) (J:2.0 Hz)	1	Ar-H; position 7
7.00 (d) (J:1.5 Hz)	1	Ar-H; position 4	7.05 (d) (J:1.5 Hz)	1	Ar-H; position 4
6.98 (d) (J:2.0 Hz)	1	Ar-H; position 5	7.00 (d) (J:2.0 Hz)	1	Ar-H; position 5
7.35 (d) (J:1.5 Hz)	1	Ar-H; position 2	7.40 (d) (J:1.5 Hz)	1	Ar-H; position 2

^a Singlets designated by (s), doublets by (d).

TABLE 2. Percent mortality of 1-day-old cockerels dosed with authentic emodin at semi-log dilutions

Dose (mg/kg)	No. of deaths/no. dosed					Total	Mortality (%)
	1/24 ^a	1/28	2/14	4/12	8/27		
37.0	0/2	1/2	4/4	5/5	5/5	15/18	83
12.2	1/2 ^b	1/2 ^b	4/4	4/5	5/5	15/18	83
3.7			4/4	0/5	3/5	7/14	50
1.2				0/5	0/5	0/10	0
0.37				0/5		0/5	0

^a Bioassay date (1974).

^b Diarrhea symptoms in survivors.

hydroxyemodin (2-hydroxymethyl-4,5,7-trihydroxy)—all reported to have UV-absorption maxima in ethanol in the range of λ 266 to 277, λ 287 to 289, and λ 430 to 452 nm (2).

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