

AFLATREM, THE TREMORGENIC MYCOTOXIN FROM *ASPERGILLUS FLAVUS*Rex T. GALLAGHER¹ & Benjamin J. WILSON²¹Applied Biochemistry Division, Department of Scientific & Industrial Research, Palmerston North, New Zealand²Center in Environmental Toxicology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232, USA

Abstract

The tremorgenic toxin isolated from strains of *Aspergillus flavus* by Wilson and Wilson in 1964, has recently been identified as an indole-mevalonate metabolite. The toxin, named aflatrem, has a remarkably similar structure to other tremorgenic toxins isolated from toxigenic *Claviceps paspali* Stevens et Hall cultures, *C. paspali* ergot, and *Penicillium paxilli* Bainier. Aflatrem is produced by some sclerotiaforming aflatoxigenic strains of *A. flavus*, and these strains may thus pose a multiple toxigenic threat.

In 1964, Wilson and Wilson (14) reported that several strains of *Aspergillus flavus* produced a tremorgenic toxin when grown on foodstuffs such as oats, millet, rice, potatoes, or corn. The purified toxin, when fed or injected intraperitoneally in small quantities into mice caused several behavioural changes, including a pronounced trembling that sometimes persisted for 3 days. Larger doses caused the initial trembling to be replaced abruptly by convulsive seizures that frequently proved fatal. Guinea pigs and rats were also susceptible to either oral or intraperitoneal administration of the toxin.

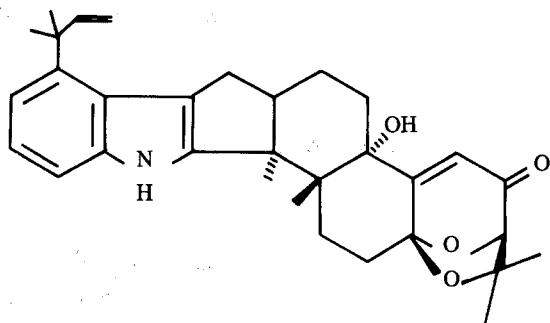
An outstanding feature of the *A. flavus* tremorgenic toxin was its ability to induce a hypersensitive state in dosed animals. Thus, mice given crude toxin via stomach tube at first became inactive, but then responded to auditory and tactile stimuli and exhibited marked tremors of the entire body when movement was attempted. At higher doses the characteristic tremors were followed within 1 to 2 hours by sudden hyperactivity with intermittent convulsions. It was observed that the slightest stimulus caused affected mice to make rapid paddling movements of the legs without forward progress. Frequently an animal would rise on its hind legs, fall backward, and make several twisting, leg-rolling motions of its entire body before righting itself. Although Wilson (15) published further information on this toxin in 1971,

interest and research on the toxin was overshadowed by investigations on the hepatotoxic and carcinogenic aflatoxin metabolites of *A. flavus* and *A. parasiticus*. That the aflatoxins are regarded as the most important mycotoxins yet discovered by man in his natural environment, is evidenced by Goldblatt's recent comments (10) that well over 2000 publications dealing with aflatoxins have appeared in the literature and many millions of dollars have been spent on research on the aflatoxins.

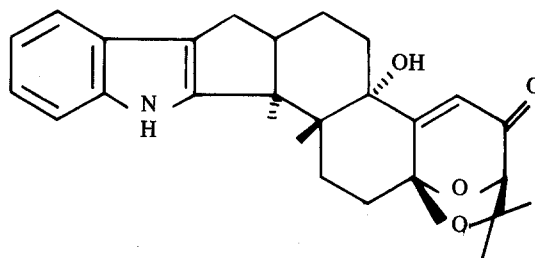
We wish to bring attention to our recent structure determination (6) of the *A. flavus* tremorgenic toxin, which we named aflatrem. Aflatrem is an indole-mevalonate metabolite with remarkable similarity to the recently isolated tremorgenic toxins, paspalinine from *Claviceps paspali* cultures and ergot (3, 7, 8), paspalitrem A from *C. paspali* ergot (3, 8), and paxilline from *Penicillium paxilli* (4, 12). The formation of such similar compounds by such diverse fungal organisms is interesting and adds a further facet to our knowledge of the fascinating *A. flavus* group of fungi.

Apart from aflatoxin and aflatrem, a number of other toxins have been isolated from various strains of *A. flavus* (2, 9, 11, 15). Perhaps most notable is the recent discovery of cyclopiazonic acid production by *A. flavus* (9, 11). A survey revealed that 28 of 54 isolates of *A. flavus* obtained from diverse sources, when grown on agricultural commodities such as wheat, rice and corn produced cyclopiazonic acid (9). Surprisingly, 14 of the isolates were found to produce both cyclopiazonic acid and aflatoxin, and this finding led to a preliminary screening of some aflatoxin-contaminated corn samples which revealed for the first time the natural occurrence of cyclopiazonic acid in agricultural commodities (9).

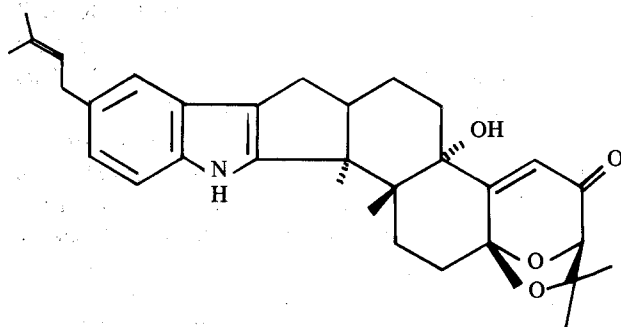
Members of the *A. flavus* group are constituents of the microflora in air and soil, and are found on or in living or dead plants and animals throughout the world (5). Strains of *A. flavus* are major storage fungi that contribute



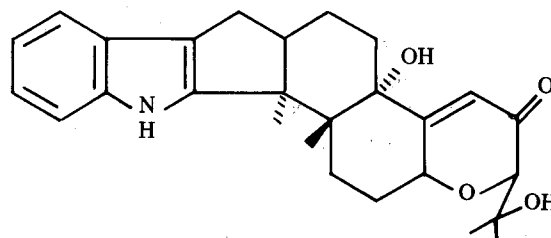
aflatrem
(*Aspergillus flavus*)



paspalinine
(*Claviceps paspali*)



paspalitrem A
(*Claviceps paspali*)



paxilline
(*Penicillium paxilli*)

to the deterioration of stored agricultural commodities, and recent investigations have shown that *A. flavus* can act as an adventitious parasite infecting commodities in the field (peanuts, corn) and orchard (almonds, walnuts, pistachio nuts, figs) (13). *A. flavus* has frequently been reported as a pathogen of man, animals and insects (1). The co-production of several toxins e.g. cyclopiiazonic acid and aflatoxin, by *A. flavus* isolates indicates that these species may pose a multiple toxigenic threat. We have found that it is strains of *A. flavus* that form sclerotia that sometimes produce aflatrem, and these strains are frequently also aflatoxigenic. Thus such strains also pose a multiple toxigenic threat, since we have shown that a substrate molded by such organisms can contain both a tremorgen (aflatrem) and a potent hepatotoxic carcinogen (aflatoxin). This possibility must be borne in mind in investigations of mycotoxicoses where aflatoxin has been

found to be present, especially when the disease syndrome is confusing, or indicative of a more complex response than might be expected from exposure to aflatoxin alone.

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