THE TOXICITY AND CHEMICAL ASSAY OF STERIGMATOCYSTIN, A CARCINOGENIC MYCOTOXIN, AND ITS ISOLATION FROM TWO NEW FUNGAL SOURCES

C. W. HOLZAPFEL,* M.SC., PH.D. (MANCH.), I. F. H. PURCHASE,† B.V.SC., M.R.C.V.S., PH.D. (CANTAB), P. S. STEYN,* M.SC., AND L. GOUWS,‡ M.SC., *Council for Scientific and Industrial Research, Pretoria*

It has been suggested that mycotoxins may play a role in the induction of liver cancer in Africa.[1] This suggestion arose out of the finding that aflatoxin B_1, a metabolite of the ubiquitous *Aspergillus flavus*, a common mould contaminant of cereals and other foods, was carcinogenic,[2-4] and was moreover the most potent hepatocarcinogen yet discovered.[5]

Sterigmatocystin, a compound which bears a close structural relationship to aflatoxin B_1,[6,7] is another mould metabolite which has been shown to be carcinogenic.[8] Hitherto, sterigmatocystin has been known only as a metabolite of *Aspergillus versicolor* (Vuillemin) Tiraboschi.[6] We have now isolated this compound from two new fungal sources and will here give a preliminary report of its toxicity to rats as established in our laboratories and a short description of a chemical assay method which we have developed.

METHODS

Several strains of fungi of local origin were grown on sterilized maize meal and the mouldy meals screened for toxicity by incorporating them in the proportion of one-quarter by weight into the feed of day-old ducklings. Those meals which proved to be toxic were extracted with chloroform-methanol and the extracts chromatographed on silica and formamide-impregnated cellulose. In 4 cases the major toxic fraction isolated by this procedure was identified as sterigmatocystin, which was present in such large quantities that it accounted for the acute toxicity of the original fungal isolates.

The acute toxicity of sterigmatocystin was studied in albino rats of 150 G body-weight from our own colony, the LD_{50} values being determined according to Weil's technique[9] on groups of 4 rats each. After preliminary trials to determine the most suitable dosage range, appropriate quantities of sterigmatocystin were administered orally in the form of saturated solutions (10 mg. sterigmatocystin per ml. of wheat germ oil and 48 mg./ml. of dimethylformamide) and intraperitoneally in the form of solutions and suspensions of varying strength (2 - 40 mg. sterigmatocystin per ml. of wheat germ oil and 48 mg./ml. of dimethylformamide). All animals which survived the test were killed on the 10th day. Controls were dosed at equivalent and higher levels with the two solvents.

*National Chemical Research Laboratory.
†National Nutrition Research Institute.
‡CSIR Microbiology Research Group.

RESULTS

Preliminary Screening for Toxicity in Ducklings

Among the moulds screened for toxicity were 5 strains of *Aspergillus nidulans* (Eidam) Wint. isolated from maize and groundnuts and one of an undescribed species of *Bipolaris* (I.M.I. 115076) isolated from animal fodder, which bore some resemblance to the *Bipolaris* state of *Cochliobolus nodulosus*. The *Bipolaris* strain and 3 of the 5 strains of *A. nidulans* caused rapid death in all the ducklings whose rations were contaminated with these moulds.

Identification of Toxic Factor in A. nidulans and Bipolaris

The major toxic fraction isolated from the meals contaminated with *Bipolaris* and the 3 toxic strains of *A. nidulans* was sterigmatocystin, which was present in extremely high concentrations (0·75 and 1·2 G/kg. in the meals infected with *A. nidulans* and *Bipolaris* respectively). The identity of this compound was confirmed by comparing it chemically with an authentic sample kindly supplied by Dr. J. C. Roberts of the University of Nottingham.

Acute Toxicity of Sterigmatocystin to Albino Rats

The LD_{50} values for sterigmatocystin administered *per os* and by intraperitoneal injection are given in Table I. In the control groups the only deaths which occurred were among rats receiving wheat germ oil in the highest oral dosage (15 ml./kg. body-weight). Death was preceded in these animals (2 out of 4 cases) by the onset of diarrhoea.

TABLE I. LD_{50} VALUES OF STERIGMATOCYSTIN IN ALBINO RATS

Solvent	*Route*	*Sex*	*LD_{50} (mg./kg.)*	*95% confidence limits*
DMF*	Per os	M	166	244 to 113
DMF	IP†	M	60	77 to 46
Wheat germ oil	Per os	F	120	155 to 92
Wheat germ oil	IP	M	65	109 to 37

*DMF = dimethylformamide
†IP = intraperitoneal

Postmortem examinations were carried out on all the animals used in the LD_{50} estimations as well as on the controls. Degenerative changes were evident in the liver and kidneys of all the rats which died from the effects of the mycotoxin, these changes being accompanied by peritonitis in the animals dosed by the intraperitoneal route. Apart from peritonitis in rats given intraperitoneal sterigmatocystin, no significant lesions were noted in the animals which survived the administration of the mycotoxin. In the control animals the only abnormalities seen were evidences of diarrhoea and slight fatty changes in the livers of the 2 rats which died.

Specimens of heart, lungs, liver and kidneys from the rats used in the LD_{50} estimations were fixed in formalin for histopathological examination. Microscopic examination of paraffin-mounted sections stained with haematoxylin and erythrocin showed varying degrees of tubular necrosis of the kidneys, usually affecting the collecting tubules only, but in some cases the convoluted tubules also. The

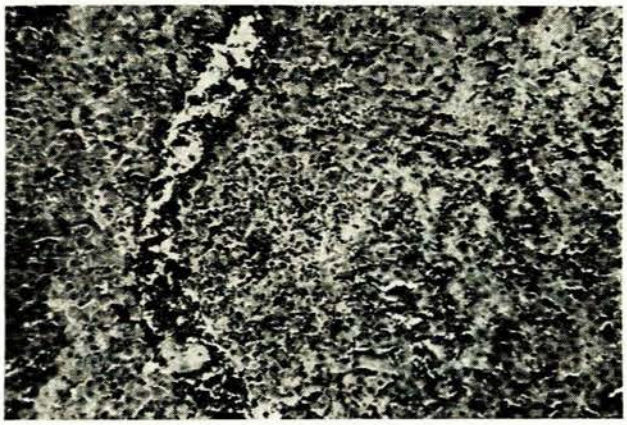

Fig. 1. Liver stained with haematoxylin and erythrocin showing centrilobular necrosis in a rat which died 3 days after oral administration of sterigmatocystin in high dosage.

liver lesions varied from a single-cell necrosis in some of the animals that survived to an extensive centrilobular necrosis in those which died (Fig. 1). Necrotic foci were encountered on the endocardial surface of the myocardium in a few cases where sterigmatocystin was given in high dosage.

Chemical Assay Method

A sample (20 G) of the material to be assayed is extracted with chloroform in a Soxhlet apparatus for 12 hours and the extract washed with water. The extract and a standard of sterigmatocystin are spotted on a silica thin-layer chromatoplate, which is then developed in chloroform-methanol (98:2) and viewed under ultraviolet light. Sterigmatocystin appears as an orange-red fluorescent spot at R_F 0·5 (limit of detection 0·5 μg.). If the developed plate is sprayed with acetic acid, sterigmatocystin exhibits a light yellow fluorescence. If the presence of sterigmatocystin is suspected on the basis of this screen test, the extract should be treated with trifluoroacetic acid and the same method of chromatography applied. The trifluoroacetic acid adduct of sterigmatocystin appears as an orange-red fluorescent spot at R_F 0·25.

DISCUSSION

The low solubility of sterigmatocystin in inert solvents presents one of the main difficulties in studying its action in biological systems. In the high dosage range, the solvents used in the oral LD_{50} determinations were being given in near-toxic quantities, and the oral LD_{50} figures obtained may therefore represent the combined effects of the toxin and the solvent. The true LD_{50} of sterigmatocystin administered by the oral route may consequently be higher than the figure reported here.

Trials with suspensions suggested that sterigmatocystin in the form of a suspension could pass through the alimentary tract and be eliminated without absorption after oral dosage. No elimination of the toxin is possible without absorption, however, after intraperitoneal injection, though it is not inconceivable that the systemic effects of the toxin might be reduced through incomplete absorption from the intraperitoneal cavity. The LD_{50} figures obtained on the basis of intraperitoneal inoculation are thus likely on the whole to be more reliable than those based on oral dosage, because the possibility of elimination without absorption precludes the use of suspensions in small, non-toxic quantities of solvent for oral administration in high dosage.

Sterigmatocystin, although much less toxic than aflatoxin and a far less potent carcinogen,[7] might nevertheless represent an important hazard to human and animal health because of the fact that it is produced in much larger quantities in contaminated meals than is aflatoxin, as was demonstrated during its isolation. It is not impossible, therefore, that in terms of its total effects sterigmatocystin might prove to be no less dangerous to the health of man and his domestic animals than the much more potent aflatoxin.

SUMMARY

The isolation of sterigmatocystin, a toxic and carcinogenic mould metabolite, from 3 strains of *A. nidulans* recovered from groundnuts and cereals and 1 strain of *Bipolaris* recovered from fodder is reported.

The acute toxicity of this mycotoxin was determined in albino rats, and the LD_{50} values obtained after oral and intraperitoneal administration are reported.

A method is described for the assay of this toxin, which, because of the widespread occurrence of the moulds responsible for its production and the large quantities in which it is elaborated, may prove to be as important a health hazard as aflatoxin despite its lower toxicity and carcinogenicity.

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