Use of a Ciliate Protozoan for Fungal Toxins Studies

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INTRODUCTION

Investigation in toxicity of unknown fungal metabolites is an important step in studies of mycotoxins. Up to now, most methods use mammalians (mice, rats), aquatic crustaceans (Daphnia, Artemia). Assays with mammalians require large quantities of substrate and these systems are usually limited in usefulness. Other technics such as tissue cultures are laborious or time consuming. Crustaceans remain the most convenient material (HARWIG and SCOTT 1971 ; EPPLEY 1974 ; JACQUET and BOUTIBONNES 1970). Bacteria, protozoa and algae have been used but never with extensive applications (HAYES et al. 1970, 1974 ; TENUISSON and ROBERTSON 1967).

In this paper, we describe the use of a ciliate protozoan *Colpidium campylum* as a valuable test for the detection of toxic fungal metabolites. We investigate its sensitivity to some known mycotoxins and to the metabolites of *Penicillium roqueforti*. Further investigations of the toxicity of some fatty acids which occur sometimes in fungal extracts (CURTIS et al. 1974) have been performed. The presented results show that *Colpidium campylum* appears to be a convenient system for testing toxicity of fungi.

MATERIALS AND METHODS

Mycotoxins

Penicillium roqueforti metabolites have been prepared as previously reported (MOREAU et al. 1976). Structure of compound 5 (Figure 1) is to be published in a chemical review. Aflatoxin B_1, Sterigmatocystin, Ochratoxin, Patulin, Diacetoxyscirpenol were purchased from MAKOR CHEMICALS. High purity (99 %) arachidonic, linoleic, linolenic,

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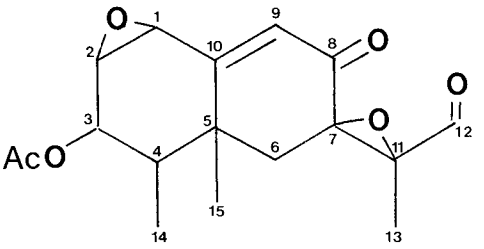
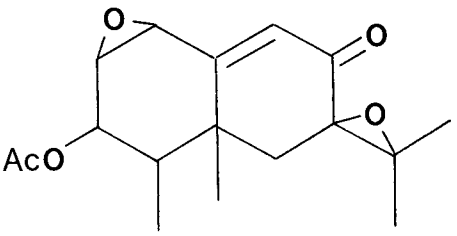
Figure 1

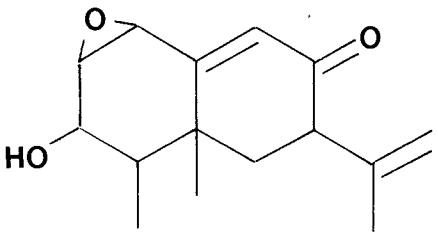
Structures of P. roqueforti metabolites



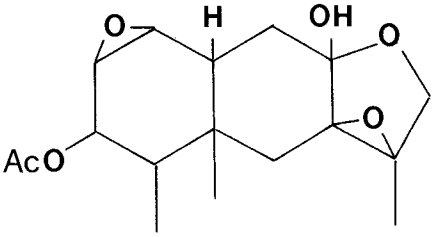
PRT 1



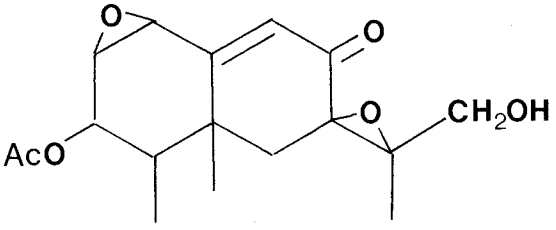
Eremofortin A 2



Eremofortin B 3



Eremofortin D 4



Eremofortin C 5

γ-linolenic and lauric acids from SIGMA CHEMICALS.

Bioassay Method

Method using *Colpidium campylum* for testing toxic compounds (mineral toxicants) has already been described in details (DIVE and LECLERC 1975, 1976, 1977). Mycotoxins were dissolved in acetone (0,25 ml) just before using, to obtain concentrations from 0,1 to 10 µg/ml in cultures. Toxicity of each solution was evaluated in five replicates. Controls were performed with each serie of tests. Cultures were allowed to grow at 20° C for 43 hours. Toxicity of mycotoxins was evaluated by means of generation number. The Minimal Active Dose (M.A.D.) is the minimal amount of toxin which modifies the growth of the culture. Concentrations above 10 µg/ml of mycotoxins have not been tested.

RESULTS

Sensitivity of *Colpidium campylum* to known mycotoxins

Table 1 shows that *C. campylum* is very sensitive to *P. roqueforti*. Toxicity is detected at the concentration of 0,25 µg/ml. The LD_{50} value abtained for *P. roqueforti* toxin (P.R.T.) is just above the M.A.D.

TABLE 1
SENSITIVITY OF *Colpidium campylum* TO SOME KNOWN MYCOTOXINS

Toxin	Minimal Active Dose* (M.A.D.) µg/ml
Aflatoxin B_1	>10
Patulin	0.5
Diacetoxyscirpenol	0.5
Ochratoxin B	>10
Sterigmatocystin	>10
Penicillium roqueforti toxin	0.25

* evaluated by means of 5 determinations

The protozoan is less sensitive to patulin and diacetoxyscirpenol. These would have been detectable only if present at a concentration of 0,5 µg/ml. Aflatoxin B_1, Ochratoxin B and Sterigmatocystin present no toxicity when tested at 10 µg/ml.

So *C. campylum* shows a wide range of sensitivity to these known mycotoxins. It is at least 40 folds more sensitive to P.R.T. and 20 folds more sensitive to patulin than to the last 3 toxins.

Toxicity of PRT and metabolites of *P. roqueforti* (Table 2)

These compounds are closely related to PRT and are extracted from the same culture of *P. roqueforti*.

TABLE 2
TOXICITY OF *Penicillium roqueforti* TOXIN AND METABOLITES OF *Penicillium roqueforti* TO *Colpidium campylum*

Compounds	M.A.D. µg/ml
PR Toxin	0.25
Eremofortin A	> 10
Eremofortin B	> 10
Eremofortin C	> 10
Eremofortin D	> 10

PR Toxin is the only metabolite to exhibit a marked toxicity in this test.

Determination of the toxicity of fatty acids to *Colpidium campylum* (Table 3)

All fatty acids which have been tested are toxic toward *C. campylum*. Arachidonic acid is highly toxic to

the protozoan. The relationship between fatty acid structure and toxicity to *C. campylum* does not parallel that reported for an other organism *Artemia salina* (CURTIS et al. 1974). In that case saturated acids with a chain length of 10-13 carbon atoms, such as lauric acid showed the greatest toxicity and oleic linoleic, linolenic acids were the most toxic of the insaturated acids. *C. campylum* does not present such a sensitivity toward these fatty acids.

TABLE 3
TOXICITY OF FATTY ACIDS TO
Colpidium campylum AND *Artemia salina*

Acid	Structure*	M.A.D.+ µg/ml	L.C.$_{50}$x µg/ml
Arachidonic acid	20:4	40	1.5.2
Linoleic acid	18:2	10	3.3
Linolenic acid	18:3	5<MAD<10	2.4
γ-Linolenic acid	18:3	5<MAD<10	
Lauric acid	12:0	5<MAD<10	5

* no of carbon atoms : no of double bonds
+ *Colpidium campylum* test
x lethal concentration assay method on the brine shrimp (*Artemia salina*) (CURTIS et al. 1974)

DISCUSSION

In the search of unknown toxic fungal metabolites the method described here cannot be considered as a screening system. When tested at concentration up to 10 µg/ml Aflatoxin B_1, Ochratoxin B, Sterigmatocystin are not detected. Aflatoxin B_1 has been shown to be degraded by an other protozoan *Tetrahymena pyriformis* (TENUISSON and ROBERTSON 1967) and so has no acute effect on population growth. This can be an explanation for the relative insen-

sibility of *Colpidium* toward Aflatoxin B_1. The use of additional biological screening systems may reduce the probability of this occurence.

C. campylum can however be useful in the detection of strains producing patulin, diacetoxyscirpenol and PR toxin.

Previous investigations had showed that some common naturally occuring fatty acids possessed toxicity toward the brine shrimp (*Artemia salina*) comparable with that of several known mycotoxins tested in that system *Colpidium* was moderately sensitive to these acids.

We have studied the toxicity of PR toxin and the related metabolites purified from a culture of *Penicillium roqueforti*. The results clearly indicate that the toxicity region of the compounds is localized and chiefly due to functions on carbon atoms 7-8-11-12-13. An acute toxicity could be attributed to the aldehyde group on carbon 12. Results also suggest that the epoxyde on carbon atoms 7-11 apparently plays a minor part in the biological activity of the compounds. So the method described here can be suitable for evaluating toxicity of compounds of a metabolic route.

To extend the possibilities of our system and to improve the technical aspects of our test we are dealing with fungal filtrates and crude extracts.

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