ABSTRACT

Modified procedures of zearalenone and moniliformin preparation, using solid substrate (rice or corn kernels) has been developed. Preliminary purification of toxins by liquid-liquid partition was applied, followed by column chromatography on silicagel and charcoal. Final yield was about 1g from 1kg of dry cultures of crystalline zearalenone and lyophilized moniliformin of high purity.

INTRODUCTION

Since the discovery at 1962 that zearalenone is a metabolite of *Fusarium* with estrogenic properties, its standard was necessary to realize surveys of cereals and feeds, to evaluate their contamination and to model experiments on its zootoxicity. Methods of standard production were described by several authors (1,2). Moniliformin was discovered by Cole et al at 1972 (3) and there is not so much data on its occurrence and significance (4,6). It is produced in high yield by *Fusarium* species, mostly by *F. avenaceum* and *F. subglutinans*, very widespread pathogens of cereals and other plants.

MATERIALS AND METHODS

BIOSYNTHESIS. For mycotoxins biosynthesis *Fusarium* strains *F. culmorum* KF.86 (ATCC 60362) *F. crookwellense* KF 232 for zearalenone production and *F. avenaceum* KF 400A, *F. subglutinans* KF 217 for moniliformin were chosen according to their high level of these metabolites produced. Biosynthesis was carried out on
solid medium: rice and corn for moniliformin and zearalenone, respectively, with water content in medium 45%, temperature 20°C.

RESULTS AND DISCUSSION

ISOLATION  A scheme of isolation procedure for zearalenone and moniliformin:

**ZEARALENONE**

**BIOSYNTHESIS**

corn kernels
KF 86, KF 232  ↓  ↓
**DRYING**

**DEPATTING**

24 hours, n-hexane
↓  ↓
**EXTRACTION**

chloroform
↓  ↓
**L-L PARTITION**

water/benzene
↓  ↓
**PURIFICATION**

silicagel column
heptane/ethanol
↓  ↓
**CRYSTALLIZATION**

methanol

**MONILIFORMIN**

rice
KF 400A, KF 217

**BIOSYNTHESIS**

**DRYING**

**DEPATTING**

24 hours, n-hexane
↓  ↓
**EXTRACTION**

methanol

**L-L PARTITION**

methanol/acetone

**PURIFICATION**

silicagel column
hexane
silicagel/charcoal column
ethyl ether
hexane
Dowex column
Charcoal column

Zearalenone:

After biosynthesis corn grains were dried in 50°C for 16 hours, then grinded and defatted in Soxhlet apparatus for 10 hours with n-hexane as a solvent. Partial migration of zearalenone to organic layer was noticed. After concentration to 300ml, organic layer was extracted with three portions of ethanol, each of 200ml. Alcohol layers were combined and concentrated. After acidification with HCl to pH=2 defatted grits were extracted with 2000ml portions of chloroform, each time the solution was evaporated nearly to dryness. Concentrated chloroform layers were added to the alcohol, evaporated to dryness and dissolved in water (200ml). Zearalenone was removed from water using benzene (4x200ml). Solvent was evaporated and about 10ml of crude oil was obtained.