Isolation of Macrocyclic and Non-macrocyclic Trichothecenes 
(Stachybotrys and Fusarium Toxins) 
from the Environment of 200 III Sport Horses

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Abstract

Satratoxins H and G, verrucarin J, and roridin E were isolated from the bedding straw of 200 sport horses exhibiting typical symptoms of stachybotryo-toxicosis. At the same time, the oat feed consumed by the horses contained non-macrocyclic Fusarium trichothecenes: T-2 toxin and diacetoxyscirpenol.

Introduction

Macrocyclic trichothecenes have been isolated from artificial Stachybotrys atra (S. atra) cultures and found to be the most important toxins of this fungus (1 - 5). These toxins are produced also in nature, and their presence was shown in the environment of calves (6), sheep (7 - 8), and humans (9) suffering from stachybotryo-toxicosis.

Herein, we report the first analytically proven case of stachybotryo-toxicosis in horses and the first natural case where both macrocyclic and non-macrocyclic trichothecene toxins were detected in the feed consumed by the animals.

Materials and Methods

Case description

In the month of August, 1983, all horses of a Budapest company (ca. 200) showed typical symptoms of experimental horse stachybotryo-toxicosis (10), especially lesions on the nose and around the mouth, and long lasting nose-bleeding. Two of the horses died, and the autopsy found small hemorrhages in the subcutis, in the muscles, and under the serosal membranes, and small lesions in the mouth. Also the workers handling the straw in a closed space suffered from long lasting nose-bleeding.
During the month of April, 1984, fleece loosing was shown by 30% of a sheep flock of Hungarian type “racka” located at another company on the Hungarian Plain. In August, 1984 the straw used for the feeding and bedding of these sheep began to be used for bedding of horses located in a stable ca. 5 kilometres from the pen of the sheep. The resulting symptoms in these horses caused by the straw were similar to those found at the first mentioned company (the retrospective study showed that the number of the affected animals from the ca. 100 horses was around 15%). Two horses died at the latter site. Autopsies revealed numerous hemorrhages in the corps, and no other causes for their deaths were evident. A third horse showed long lasting nose-bleeding.

The straw was heavily contaminated by *S. atra* at both companies. The contaminated feed (oats and hay) and bedding (straw) was removed, and no new animals became affected. The ill horses were treated with topical ointments and appeared to recover. However, in the following week, rhinopneumonitis infectiosa equorum in a serious form showed up in many of the animals of the second company.

**Sample analysis**

Straw from both places was analysed essentially as described earlier (4, 7). Briefly, 100 g straw samples were extracted twice with methanol. After vacuum concentration, the resulting residue was partitioned between 100 mL of acetonitrile and 100 mL of petroleum ether (bp 40 – 70°C). The acetonitrile layer was concentrated, taken up in 5 mL of methylene chloride, layered on a silica gel column of 15 g (Kieselgel 60, 0.2 – 0.5 mm, 35 – 70 mesh, Merck). The toxins were eluted with 100 mL of ethyl acetate, concentrated and analysed by high-pressure liquid chromatography (HPLC): Waters 6000A apparatus, Polygosil 60-D 5 C18 column (Macherey-Nagel), gradient elution (20 – 80% of methanol).

The peaks which eluated at identical retention volumes with those of the standards were collected and evaporated under N2 stream. Then, 200 μL of 0.5 M sodium methoxide was added, and the mixture was heated for 15 min at 60°C. After neutralization of the sample with 250 μL of 0.5 N HCl in methanol, the sample was dried under N2. N-O-bis(trimethylsilyl)trifluoroacetamide (Pierce Chemicals) reagent (50 μL) was added to the sample (60°C for 15 min). After evaporation under an N2 stream, it was analyzed for the presence and quantity of verrucarol (the common transesterification product of all known *Stachybotrys* toxins) by capillary gas chromatography (GC): Packard 427 with flame ionization detector and HP3390/A integrator, 14 m x 0.30 mm column with SE-52 (Supelco Inc) stationary phase, 140 – 220°C, 4°C/min. The quantification of the verrucarol was done by comparing its GC peak with that of a standard, and the gained values served as a basis to calculate the amount of the macrocyclic trichothecene in each of the original HPLC peaks (e.g. 1 μg of satratoxin H is equivalent with 0.48 μg of verrucarol).

The quantitative and qualitative analysis of the oat sample from the first company for *Fusarium* toxins was done by capillary gas chromatography as described earlier (11).

**Results and Discussion**

HPLC and GC analyses showed that the straw used for bedding the horses contained *Stachybotrys* toxins in the concentration of 75 μg/kg satratoxin H, 35 μg/kg verrucarin J, 20 μg/kg satratoxin G, and about 5 μg/kg roridin E for the most contaminated of the samples obtained from the first company (Fig.1), and 107 μg/kg satratoxin H, 61 μg/kg verrucarin J, and 35 μg/kg satratoxin G for one straw sample from the second company. (Altogether 5 straw samples obtained from the first company and 2 from the second were analysed and all were positive.)