Ammoniation of Aflatoxin B_1 in a Pressure Chamber Used to Decontaminate Toxin-Containing Cottonseed Meal

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ABSTRACT

Radiolabelled aflatoxin B_1 mixed with non-labelled B_1 distributed on an inert carrier was treated in a pressurized ammoniation chamber with 4% ammonia at 40 psi and held at 100 C for 30 min. Twenty per cent of the radiolabel was lost, probably as volatiles. Less than 1% of the original toxin was recovered as B_1 indicating that ammoniation altered the structure of essentially all of the B_1. Approximately 20% of degraded aflatoxin B_1 was accounted for as a 206 MW compound that exhibited properties of a nonfluorescent phenol with a difuran moiety but neither the lactone carbonyl nor the cyclopentenone ring of aflatoxin B_1. The remaining degradation products were fragments of B_1 having molecular weights less than 200.

INTRODUCTION

Meal produced from the processing of cottonseed infected with *Aspergillus flavus* can be contaminated with aflatoxins. One of the most promising practical approaches to detoxification is treatment with ammonia gas at elevated temperatures under pressure (1,2). Aflatoxin in such contaminated meals exists as a component of the meal matrix, and other meal components could influence toxin degradation. To study the chemistry of detoxification a model investigation was conducted that involved aflatoxin B_1 ammoniation with ammonium hydroxide in the absence of meal (3,4). Two degradation products were formed: I, 286 MW and II, 206 MW (Fig. 1). Since the model system utilized ammonium hydroxide rather than ammonia gas, high moisture, and a non-regulated chamber, the present study was undertaken to determine the products formed by ammoniation of B_1 in a system that utilized ammonia gas and monitored pressure in pilot-scale equipment previously used to reduce aflatoxin in a large sample of naturally contaminated cottonseed meal from 4000 to 4 μg/kg (5).

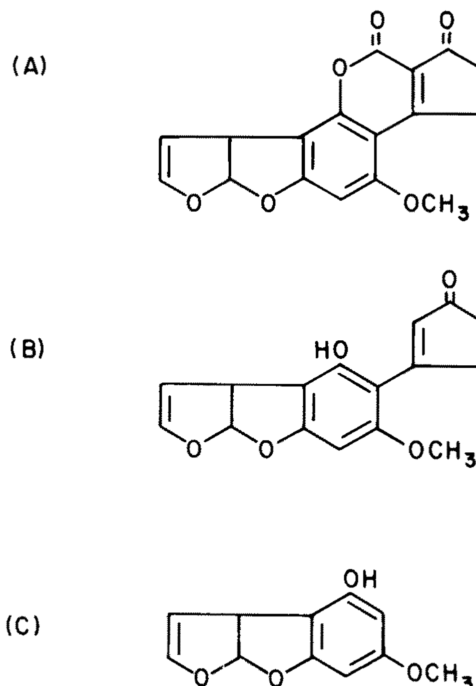

FIG. 1. Structures of aflatoxin B_1 and of products formed from ammoniation of aflatoxin B_1 in model systems. (A) Aflatoxin B_1, MW 312. (B) I. MW 286 formed using NH_4OH as the ammonia source. (C) II. MW 206 formed using both NH_4OH and ammonia gas as the ammonia source.

MATERIALS AND METHODS

Crystalline aflatoxin B_1 (200 mg) and 5 μCi of uniformly labelled aflatoxin B_1 (spec. activity 50 mCi/mmol) were dissolved in methylene chloride and distributed by pipette onto ca. 5 g of silica gel H. After air-drying overnight to remove solvent, the gel was transferred to a square of fine mesh nylon cloth secured as a small bag fastened with a twist of stainless steel wire. The bag was placed inside a polyethylene bottle (4 cm diam. and 10 cm high) that had been punctured on the top and sides to allow permeation by ammonia gas. The bottle was strapped to the shaft of the ammoniator described in earlier reports of pilot-scale detoxification of oilseed meals (2).

Anhydrous ammonia to produce 4% ammonia gas was delivered by a technique described by Koltun et al. (6). Pressure and temperature were mechanically monitored. The ammoniator was charged with ca. 50 lb of cottonseed meal. Ammoniation was conducted at 100 C for 30 min at 40 psi (5).

After air-drying the gel was transferred to a 150 ml coarse (60 μm pores) fritted glass filter funnel. Solubles were eluted sequentially with an eluotropic series of solvents; extracts were collected in tared beakers, solvents evaporated and dried extracts weighed. Elution was carried out with cyclohexane, methylene chloride, acetonitrile, methanol and finally with 10% acetic acid in methanol. From 200 to 300 ml of each solvent were used. Radioactivity was measured using previously described equipment, techniques and solvents (7). Activity was measured on the starting material and on aliquots of each fraction. All fractions were monitored by thin layer chromatography (TLC); plates were viewed in ultraviolet (UV) and in daylight after spraying with Fast Blue Salt B, a coupling reagent for visualizing phenols (8). Fractions also were monitored by mass spectroscopy on a Finnigan 1000 GC/MS. Preparative TLC with Adsorbosil-1 plates and chloroform: acetone (9:1) as a developing solvent was used to purify material in zones of interest.

RESULTS AND DISCUSSION

Approximately 20% of the original radiolabel was lost during ammoniation. Although experimental conditions did not allow for trapping of carbon dioxide, previous studies (7) demonstrated cleavage of the lactone ring of B_1 with release of carbon dioxide (3). This mechanism could account for part of the label loss. Other small MW volatile fragments may have been produced. Weight per cent of fractions closely paralleled per cent of radioactivity. Label was distributed in proportion to the mass eluted with each solvent. Five mg of degradation product was cyclohexane-soluble, 22.5 mg was soluble in benzene, 35 mg in methylene chloride, 25 mg in acetone, 1.2 mg in acetonitrile, 47.2 mg in methanol and 5.2 mg was eluted with acidic methanol. Of the total 141.1 mg degradation product eluted, only 0.42 mg was accounted for as unreacted

aflatoxin B_1. Benzene, methylene chloride, acetone and acetonitrile all eluted some of the unchanged toxin, the greatest amount (0.365 mg) being methylene chloride-soluble.

Benzene and the first wash with methylene chloride eluted fractions containing a compound that stained red with Fast Blue Salt B (8). Preparative TLC of the fractions containing this compound yielded nearly 28 mg of crystalline material. Identity of the compound was confirmed as II (Fig. 1), a nonfluorescent phenol, 206 MW, that retains the difuran moiety but lacks both the lactone carbonyl and the cyclopentenone ring of aflatoxin B_1. The spectrum of the purified material was identical to that reported previously for II (4). No compounds in these two fractions other than B_1 and II were detected by gas chromatography-mass spectroscopy (GC-MS). All other fractions contained fragments with molecular weights less than 200.

In the model study of Cucullu et al. (4) using ammonium hydroxide as the ammonia source conversion of B_1 to I and II (Fig. 1) after 1 hr ammoniation were 11% and 3% respectively, and after 3 hr, 19% and 10%. Moreover, when I (286 MW) was ammoniated with ammonium hydroxide no II (206 MW) was formed (9); therefore, II must be formed from B_1 rather than from I. In the model ammoniation reported here, compound II was formed but I was not detected. Twenty-eight mg of II was purified from fractions eluted with benzene and methylene chloride. Since the fractions containing II as the major component comprised 43.5 mg of the 141.1 mg reaction product recovered, the conversion of B_1 to II was greater than 20%. Of importance is the apparent volatilization of some degradation products and the lack of formation of I during ammoniation using ammonia gas rather than ammonium hydroxide. It is assumed that, as in the formation of I (286 MW), the 206 MW compound also arises from opening of the lactone ring of B_1 followed by decarboxylation (3).

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