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Reduction in Alkaloid Content of Ergot Sclerotia by Chemical and Physical Treatment

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Total alkaloid contents of wheat ergot sclerotia were reduced greatly (ca. 90%) within several hours by treatment with chlorine and heat (150 and 200 °C) but only slightly (<20%) by sulfur dioxide and hydrogen chloride. Ammonia, ozone, and ultraviolet irradiation had no significant effect on alkaloid levels. The rates of decomposition by chlorine were the same for the five pure alkaloids studied. Peptide alkaloids with the amide linkage β to the ergoline ring were rapidly converted to the α configuration prior to thermal degradation. A reduced toxic effect by ergot on growing chickens was observed after the sclerotia were autoclaved at 121 °C for 30 min.

The presence of mycotoxins in animal feeds can lead to reduced growth, feed utilization, and resistance to disease and also to increased pathological disorders and to residual toxins in the tissues of animals destined for human consumption (Wyllie and Morehouse, 1978). Methods that reduce the toxicity of animal feeds containing mycotoxins would enable feed manufacturers to utilize safely mycotoxin-contaminated feeds that might otherwise have to be destroyed or used in such a manner that the economic return to the farmer is reduced. Such methods could also result in a greater production of cereals that tend to be susceptible to mycotoxin contamination. Some grains containing aflatoxin and the fungi that produce it have been detoxified and/or decontaminated with ammonia (Jemmali, 1980).

Ideally, detoxification methods for mycotoxins should be effective, inexpensive, and simple, use existing technology, and not alter nutritional and other parameters of feed constituents. Chemical methods include treatment with gases, such as ammonia, chlorine, hydrogen chloride, ozone, and sulfur dioxide, or with solutions such as bleach, dilute acid or alkali, and hydrogen peroxide. Physical methods include treatment with heat or ultraviolet (UV) light or physical separation from seeds on the basis of differences in density or size.

Sclerotia of the fungus *Claviceps purpurea* (Fr.) Tul. (ergot) that are larger than the grain kernels can be physically separated by sieving (Seaman, 1980). Alternatively, ergot can be removed by flotation in a brine solution (Seaman, 1980), although this results in damp salty grain. Prompted by reports that pelleting of ergot-contaminated feeds reduced the toxicity of such feeds to swine (Friend and Macintyre, 1969) and poultry (Bragg et al., 1970; O'Neil, 1980), various treatments were investigated to determine if any might show promise for detoxification of animal feeds. In addition, the effect of autoclaving on the toxicity of ergot fed to poultry was studied.

EXPERIMENTAL SECTION

Materials. Wheat ergot was obtained from Northern Sales, Ltd., Winnipeg, Manitoba, reactant gases were from Matheson, and ergot alkaloid standards were from Sandoz AG, Basel, Switzerland.

Treatment of Ergot Sclerotia. Typically, 30 whole sclerotia of comparable size and weighing ca. 3 g in total were placed in a 500-mL round-bottom flask. The flask was evacuated under vacuum, filled with the treatment gas, reevacuated, and refilled to ambient pressure and temperature. After being allowed to stand in the dark at room temperature for the the treatment period, the sealed flask was evacuated and flushed well with air. Ozone (4.4 mol %) was generated in pure oxygen and passed over the sclerotia at a flow rate of 20 mL/min. For other treatments, the sclerotia were placed into an oven at the desired temperature or into a special ultraviolet irradiation chamber (Young, 1976). In several instances, the sclerotia were ground to a powder before treatment.

Extraction of Sclerotia. Sclerotia were ground in a mortar, and a 200-mg aliquot was defatted by extraction with hexane in a Goldfisch apparatus for 4 h. The residue was extracted exhaustively with 1 N H_2SO_4–acetonitrile (1:1) (3 × 5 mL). The combined acid extracts were readjusted to ca. pH 9.6 with the addition of concentrated NH_4OH(ca. 4 mL), the basic solution was extracted with chloroform (1 × 5 mL, 2 × 2.5 mL), and the combined organic extracts were dried over $MgSO_4$, filtered, concentrated under reduced pressure, and made up to exactly 10.0 mL with chloroform prior to analysis. All extractions were done under subdued light or with the use of low actinic ware glass apparatus.

Alkaloid Analysis. Total alkaloid contents of the extracts were determined by use of the modified van Urk reagent (Young, 1981a). Individual alkaloid compositions were determined by high-performance liquid chromatography (HPLC) using a Waters Scientific Radial-PAK cartridge packed with μBondapak RP-18 (10 μm) coupled to a Waters Scientific Model 420 fluorescence detector with a F4T5/BL lamp, 360-nm excitation filter, and 425-nm emission filter. The column was eluted at 2.0 mL/min with a linear gradient over 20 min from 100:0 to 60:40 of aqueous ammonium carbonate (200 mg/L)–acetonitrile–methanol (55:30:15) and acetonitrile–methanol (2:1). A typical reversed-phase HPLC chromatogram of alkaloids in Canadian ergot sclerotia has been described elsewhere (Young, 1981a), as have the response characteristics of this fluorescence detector (Young, 1981b).

Treatment of Ergot Alkaloids. A 200-μL aliquot of a stock solution containing ergometrine (ergonovine) (5.13 μg/mL), ergosine (21.6 μg/mL), ergotamine (20.5 μg/mL), ergocornine (30.5 μg/mL), and ergocryptine (21.6 μg/mL) was placed into a 25-mL round-bottomed flask, taken to dryness on a rotary evaporator, and treated with various

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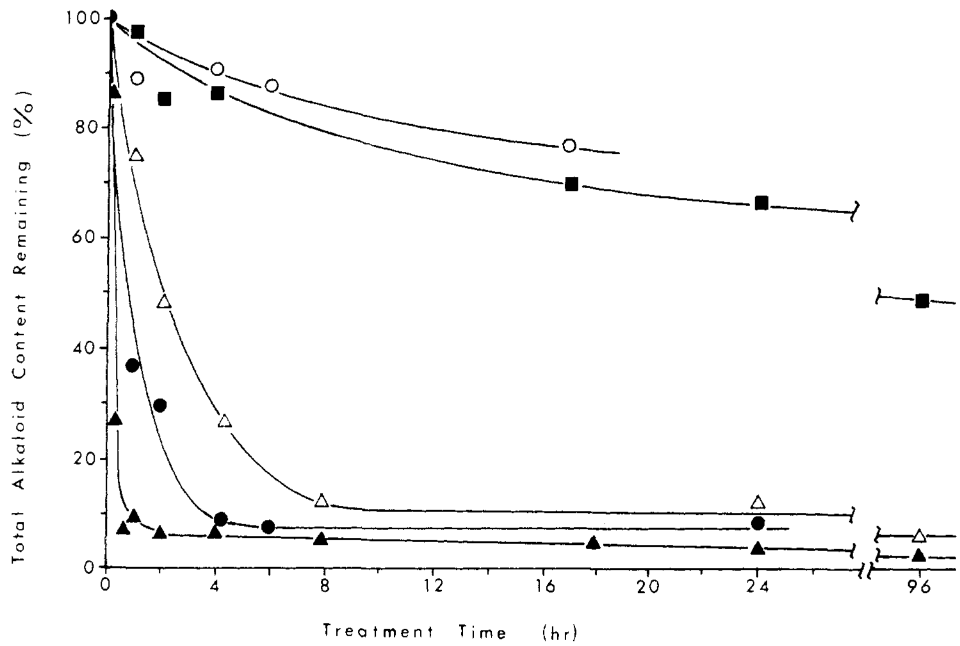

Figure 1. Reduction in total alkaloid content of wheat ergot sclerotia by chemical and thermal treatment. Treatments: (●) chlorine; (■) hydrogen chloride; (○) sulfur dioxide; (△) 150 °C; (▲) 200 °C.

gases as above. The flask was purged with air and rinsed with 500 μL of methanol, and an aliquot was analyzed by HPLC.

Effect on Alkaloid Content and Toxicity to Chickens of Autoclaving Ergot Sclerotia. Ergot sclertia were finely ground and autoclaved moist at 121 °C for 30 min. The treated and untreated ergots were added to the chicken diet at the 4% level, by weight, and fed to week-old male leghorn chicks for 7 days. There were five chicks per cage and four cages per treatment. Experimental design and other treatments are reported elsewhere (Young and Marquardt, 1982). For alkaloid analysis, a mixture of 0.5 g of feed, 5 mL of hexane, and 5 mL of 1 N H_2SO_4–acetonitrile (1:1) was homogenized (Polytron Type PT 10 20 350D, Brinkmann Instruments, Rexdale, Ontario) for 30 s and centrifuged, and the hexane layer was removed. The remaining supernatant was removed and the residue reextracted with H_2SO_4–acetonitrile (3 × 5 mL). Combined extracts were concentrated under reduced pressure at room temperature to remove acetonitrile, made slightly alkaline (ca. pH 9) with concentrated NH_4OH, and centrifuged, and the aqueous layer was removed. The residue was extracted with water (2 mL). The combined aqueous extracts were adsorbed on a Waters Scientific C18 Sep-PAK cartridge, which had been previously activated with methanol (5 mL) and water (5 mL), and eluted with water (3 mL) and methanol (10 mL). Aliquots were analyzed for total and individual alkaloid contents as described above.

RESULTS AND DISCUSSION

The results of various treatments that were investigated for their ability to reduce the alkaloid content of wheat ergot sclerotia are shown in Figure 1. Chlorine and heat treatment (150 and 200 °C) had the greatest effect, with 90% reductions occurring within 4 h. Sulfur dioxide and hydrogen chloride had only moderate effects. Ammonia (24-h treatment) and ozone (16 h) had no significant effect on alkaloid content or composition.

The rates of reaction of ergot alkaloids with chlorine were much more rapid for the pure alkaloids on a glass surface than for alkaloids contained in sclerotia. In 100% chlorine, pure alkaloids decomposed completely within several minutes, whereas alkaloids contained in sclerotia

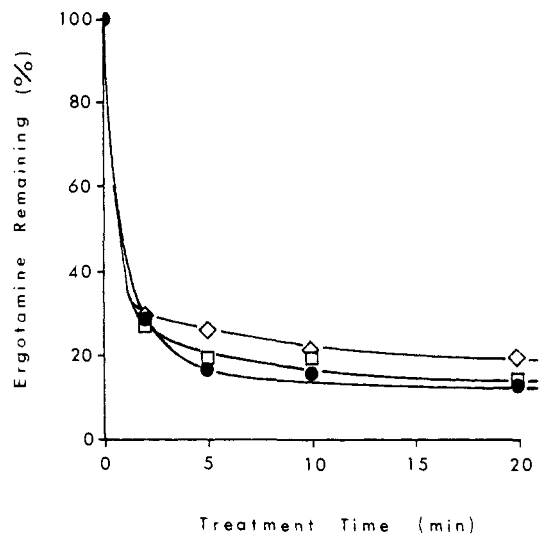

Figure 2. Effect of various concentrations of chlorine on ergotamine. Chlorine concentration in nitrogen: (●) 1%; (□) 0.24%; (◇) 0.032%. Effects on ergocornine, ergocryptine, ergosine, and ergometrine were not shown as they were identical.

required about 4 h for 90% destruction, probably because chlorine had to diffuse into the body of the sclerotia before reaction could occur. Even at low chlorine concentrations (0.032%), the decomposition of pure alkaloids proceeded rapidly (Figure 2). The rates of reaction with chlorine were the same for all the alkaloids studied (ergocornine, ergocryptine, ergometrine, ergosine, and ergotamine), whether pure or contained in sclerotia.

The total alkaloid content of the wheat ergot used in this study was 0.357%, by weight, and consisted of ergocornine (9.7%), ergocorninine, (1.3%), ergocristine (30.1%), ergocristinine (2.2%), ergocryptine (6.6%), ergocryptinine (0.9%), ergometrine (21.3%), ergometrinine (9.1%), ergosine (4.9%), ergotamine (12.2%), and ergotaminine (1.7%).

When heated to 200 °C, the ergot alkaloids with the amide linkage in the normal β configuration (-ine) appeared to be rapidly converted to the α configuration (-inine) prior to thermal degradation. Within 10 min, the

Table I. Effect of Autoclaved Wheat Ergot Sclerotia on the Performance of Male Leghorn Chicks after Seven Days of Feeding

dietary wheat ergot, %	feed consumed, g	weight gained, g	feed/gain ratio
0	114.1[a] ± 1.1a[b]	61.1 ± 0.9a	1.87 ± 0.02a
4 (autoclaved)	78.9 ± 2.8b	29.4 ± 1.8b	2.68 ± 0.09b
4	61.6 ± 1.5c	13.9 ± 1.0c	4.52 ± 0.36c

[a] Mean ± standard error per bird for five birds per pen and four pens per treatment. [b] Means in the same column with different letters differ significantly at $P < 0.05$ according to the Student-Newman-Keuls multiple range test.

percentage of the α isomers in all the peptide alkaloids (ergocornine, ergocristine, ergocryptine, ergosine, and ergotamine) taken together increased from 9% to 19% while the total amount of these alkaloids remained about the same; after 40 min the percentage had increased to 41% while the total amount decreased. The simpler nonpeptide alkaloid ergometrine did not undergo this isomerization to any great extent prior to decomposition.

Ergot alkaloids are photosensitive (Stoll and Schlientz, 1955); however, irradiation of ground sclerotia with UV light for 54 h did not alter total alkaloid levels or individual alkaloid composition. The light would not be expected to penetrate very far into the sclerotial tissue, although Silber and Bischoff (1954) reported that alkaloids are concentrated in the outer layers and levels decrease toward the center.

Autoclaving ergot sclerotia at 121 °C for 30 min resulted in a 24.6% reduction in total alkaloid content. The treated ergot had a reduced toxic effect on growing chicks (Table I). Toxic effects, such as reduced feed consumption and weight gain, are directly proportional to the alkaloid levels in the diet (Young and Marquardt, 1982); the observed feed consumption and weight gain values are in reasonable agreement with those calculated (74.5 and 25.5 g, respectively) for ergot with a 24.6% reduced alkaloid content. Another benefit of autoclaving was a significant improvement in the feed/gain ratio (Table I).

Heat generated in the feed pelleting process probably reduces the alkaloid content in ergot-contaminated feed and accounts for the reduced toxicity of such treated feeds (Friend and Macintyre, 1969; Bragg et al., 1970; O'Neil, 1980).

The results of these experiments show that detoxification of ergot sclerotia is possible. Further studies are under way to establish the practicality of these findings.

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Registry No. Ergometrine, 60-79-7; ergosine, 561-94-4; ergotamine, 113-15-5; ergocornine, 564-36-3; ergocryptine, 511-09-1; ergocorninine, 564-37-4; ergocristine, 511-08-0; ergocristinine, 511-07-9; ergocryptinine, 511-10-4; ergometrinine, 479-00-5; ergotaminine, 639-81-6; chlorine, 7782-50-5; sulfur dioxide, 7446-09-5; hydrogen chloride, 7647-01-0.

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Effects of Processing on the Sodium:Potassium and Calcium:Phosphorus Content in Foods

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The effects of processing on sodium:potassium (Na:K) and calcium:phosphorus (Ca:P) ratios in peanuts, wheat, tuna, canned green beans, whole kernel and cream-style corn, carrots, peaches, frozen green beans, corn, broccoli, cauliflower, and french fried potatoes were studied. Minerals were determined in samples taken at various stages during processing. Processing of canned vegetables caused a loss in phosphorus and an increase in the Na:K ratios, when salt was added. Freezing vegetables had very little effect on the minerals studied. Processing of peanuts resulted in an increase in Na:K ratios in peanut butter. Mineral content of whole wheat flour was unaffected by milling, whereas the potassium and phosphorus content of white flour was reduced. In tuna, canning in either oil or water affected mineral values.

Many studies have been conducted over the years on the nutrient composition of processed foods. The majority of these studied have been concerned with the losses of water-soluble vitamins during processing. Few studies have been conducted on the effect of processing on the mineral content.

General commercial processes which may result in mineral losses include peeling, blanching, and cooking (Fennema, 1976). Losses of minerals due to peeling or trimming of fruits and vegetables may result due to une-

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