A Method for the Detection of Ergot Contamination in Ground Triticale Grain

James E. Robbers, Veronica M. Krupinski, Hadi S. Sheriat, and Donald M. Huber

Associate Professor, Research Fellow, Research Fellow, Department of Medicinal Chemistry and Pharmacognosy; and Associate Professor, Department of Botany and Plant Pathology, respectively, Purdue University, West Lafayette, Indiana 47907.

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ABSTRACT

Utilizing *Triticale* ergot, official ergot (National Formulary XI) and *Triticale* grain, a reliable method was developed for the detection of ergot contamination in ground grain. The method involves the extraction of the ergot alkaloids from the ergoty grain using solvent partitioning procedures, and then quantitatively determining the amount of alkaloid present by using a colorimetric assay. Thin-layer chromatography showed that *Triticale* ergot contains numerous alkaloids including the toxic compounds, ergonovine and ergocristine.

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Triticale is an artificial genus containing amphiploids resulting from the hybridization of wheat and rye (4). These hybrids were formed to combine the yield, milling, and baking characteristics of wheat with the hardiness of rye (6), and its adaptability to production on marginal land or in northern latitudes not suitable for corn or sorghum. Triticales strains currently produced are not suitable for bread flour; however, they have been evaluated experimentally as a feed grain for livestock (3, 8, 10).

A distinct disadvantage of *Triticale* as a feed grain is its susceptibility to attack by the ergot fungus. Barger (2) states that the greater susceptibility of rye to ergot infection is based on the fact that rye, unlike wheat and barley, depends largely on cross-fertilization, and opens its glumes in order to receive pollen from other plants. After fertilization the glumes are again closed. However, if adverse weather prevents pollination from occurring, the glumes remain open much longer and increase the risk of ergot infection. Unfortunately *Triticale* more nearly resembles the rye parent than wheat in this characteristic, and it is therefore more susceptible to ergot infection than wheat.

Indications are that *Triticale* ergot is toxic, since Bragg et al. (5) have determined that 1.6% *Triticale* ergot in the diets of broiler chicks significantly depressed growth and increased the feed per gain ratio. At double the level (3.2%), a 100% mortality resulted at 48 days of age. Ingalls et al. (10) mentioned that a possible cause for reduced intake of *Triticale* diets in young dairy calves may have been due to the fact that the grain was contaminated with ergot.

Classically, the percentage of ergot in a grain sample is determined by taking a random sample of the grain, and then manually separating the dark purple ergot sclerotia from the grain. This type of sampling indicates only that ergot is present, but does not provide any information on the amount or the types of alkaloids present (7). This is a particular problem when one realizes that the toxicity of ergot is due to the presence of certain alkaloids of the peptide type such as ergotamine; therefore, a determination of the amount of sclerotia in a grain sample is not necessarily a true indication of the toxic potential of the grain. Also, the sampling cannot be used with prepared feeds in which the grain has been ground, since the sclerotia cannot be detected visually. The purpose of this investigation was to devise a reliable method to determine quantitatively ergot alkaloid contamination in ground *Triticale* grain and to identify the major alkaloids present.

MATERIALS AND METHODS.—*Alkaloid extraction from ergot sclerotia and ergot-grain mixtures.*—Samples (500-mg) of ergot sclerotia obtained from *Triticale* and of "official" ergot [National Formulary XI, which is defined as the dried sclerotium of *Claviceps purpurea* (Fries) Tulasne developed on rye plants] were ground to 0.97 mm (20-mesh) in a Wiley mill and defatted by shaking on a rotary shaker for 1 hour with two successive 50-ml portions of petroleum ether, b.p. 30-60.

The defatted ergot samples were suspended in 30 ml of a mixture of chloroform-methanol-NH_4OH (90:9:1, v/v) and shaken on a rotary shaker for 5 minutes. Then 15 ml of chloroform and 1.0 ml of water were added and the suspension was shaken for another 5 minutes. After filtering by suction through a sintered glass funnel, the ergot samples were repeatedly extracted with 30-ml portions of chloroform-methanol NH_4OH (90:9:1, v/v). Each extraction was tested for alkaloids using the colorimetric assay and the extractions were continued until the solvent no longer removed alkaloids. For the colorimetric assay, all extracts including the petroleum ether extracts from the defatting procedure, were kept separate and evaporated to dryness (not over 40 C) using a flash evaporator. Each residue was taken up in 100 ml of ether (USP). The ether was decanted and the flask rinsed with 5 ml of 95% ethanol. The ethanolic and ethereal fractions were combined, and the solution shaken with four 25-ml portions of aqueous 2% succinic acid. The aqueous fractions were combined and made alkaline (pH~11) with concentrated NH_4OH. After the alkaline aqueous solution was extracted with four 30-ml portions of chloroform, the combined chloroform extract was evaporated to dryness using a flash evaporator and

alkaloid quantitation was performed directly on the residue.

Additional extraction of the same ergot samples was performed by shaking for 10 minutes with 30-ml portions of chloroform. Each chloroform extract was evaporated to dryness and the residue treated as in the extractions with chloroform-methanol-NH_4OH. The extractions were continued until the sclerotia were exhausted of alkaloids.

Measured amounts (350 mg) powdered *Triticale* ergot and "official" ergot were mixed with 100 g of powdered *Triticale* grain (Illinois-grown, Fasgro, cultivar 204). The extraction procedure was identical to that used to extract ergot sclerotia with the exception that the defatting and alkaloid extracting procedures were performed with 200 ml of solvent and the mixtures were shaken for 20 minutes.

Colorimetric assay for alkaloids.—All extracts were evaporated to dryness and 1.0 ml of a 2% succinic acid solution was added, followed by 2 ml of modified van Urk's reagent (1, 12). Modified van Urk's reagent was prepared by mixing 65 ml of concentrated H_2SO_4 with 35 ml distilled water, cooling, and adding 200 mg of *p*-dimethylaminobenzaldehyde, and 0.15 ml of 10% $FeCl_3$ solution. The *p*-dimethylaminobenzaldehyde produces a blue color with ergot alkaloids as a result of reacting with the carbon atom in the α-position relative to the cyclic NH-group of the indole portion of the alkaloid molecule. The solution should stand for 20 minutes to allow the color to develop. The intensity of the color is measured as absorbance at 580 nm against a blank containing 1.0 ml of 2% succinic acid and 2 ml of van Urk's reagent. The total alkaloid, calculated as ergonovine, is determined by multiplying the absorbance by 85.8 which is the reciprocal of the slope of a standard curve based on ergonovine.

Identification of alkaloids in ergot sclerotia grown on Triticale.—*Triticale* sclerotia (55.70 g) which passed a 246 μm (60-mesh) screen were extracted with 20 ml 2% succinic acid by shaking for 1.0 hour. The aqueous extract was removed by suction filtration, and the extraction repeated until no additional alkaloid was extracted as indicated by the colorimetric assay.

The combined succinic acid extracts were brought to pH~11 with concentrated NH_4OH and the water-insoluble alkaloids were extracted with five 100-ml portions of $CHCl_3$. The aqueous portion was made neutral with concentrated H_2SO_4, then saturated with NaCl. Water-soluble alkaloids were extracted with five 100-ml portions of $CHCl_3$. Both $CHCl_3$ extracts were dried by filtration through anhydrous Na_2SO_4, and were evaporated to several milliliters each on a flash evaporator.

Thin-layer chromatography of Triticale alkaloid extracts.—Both the water-insoluble and water-soluble alkaloid extracts were chromatographed on Bakerflex silica gel plates in acetone-chloroform (4:1). After development, the plates were sprayed with Ehrlich's reagent (1.0 g *p*-dimethylaminobenzaldehyde, 10 ml water, and 20 ml concentrated HCl). The extract containing water-insoluble alkaloids showed eight Ehrlich-positive (blue-purple) spots, while six Ehrlich-positive spots were visible in the extract containing water-soluble alkaloids.

Both extracts were then chromatographed on silica gel G preparative thin-layer chromatography plates (0.25 × 200 × 200 mm) in acetone-chloroform (4:1, v/v). Each Ehrlich-positive band, as indicated by spraying the edge of the plate, was scraped from the plate, and the silica gel extracted with four 50-ml portions of methanol by shaking for one hour on a rotary shaker. The methanol extracts were filtered through sintered glass funnels, and taken down to a few milliliters on the flash evaporator. For identification of the alkaloid components, each extract was chromatographed two-dimensionally along with reference alkaloids on Bakerflex plates using acetone-chloroform (4:1, v/v) and acetone-ethylacetate-dimethylformamide (5:5:1, v/v).

RESULTS AND DISCUSSION.—Official ergot (National Formulary XI) was employed in this

TABLE 1. Determination of the amount of alkaloid extracted from 500 mg of ergot sclerotia samples expressed as μg/g of dry weight of sclerotia

Extract	Official ergot (NF XI)[a]	*Triticale* ergot
Petroleum ether	485[b]	490
Chloroform-methanol-NH_4OH (90:9:1)	4,936	1,625
Chloroform	81	62
Total	5,502	2,177

[a]The dried sclerotium of *Claviceps purpurea* developed on plants of rye, *Secale cereale* which yields not less than 0.15% of total alkaloids.

[b]Total alkaloid from all extracts determined as ergonovine.

TABLE 2. Efficiency of extraction procedure in recovering alkaloid from 100 g of finely-ground *Triticale* grain[a] contaminated with 0.35% ergot sclerotia

Sample	Total added (μg)	Amount (μg) of alkaloid extracted: Petroleum ether	Chloroform-methanol-NH_4OH (90:9:1)	Chloroform	Total	Recovery (%)
Official Ergot (NF XI) plus *Triticale* grain	1,926	153	1,618	64	1,835	95.3
Triticale ergot plus *Triticale* grain	762	12	659	51	722	94.8

[a]Illinois-grown, Fasgro, cultivar 204.

investigation to serve as a standard because the chemistry and toxicity of the alkaloids present in the ergot are well known, whereas the *Triticale* ergot has not previously been investigated chemically. The National Formulary XI sets for ergot a lower limit for total alkaloid of not less than 0.15%, but no upper limit has been set. Therefore, the general approach to the extraction problem was to first determine the absolute amount of alkaloid present in the ergot samples by exhaustive extraction and then quantitation. This standardization of the ergot sclerotia is shown in Table 1. The official ergot contained 0.55% total alkaloid, and even though the *Triticale* ergot contained approximately one-half the amount of alkaloid (0.22%) as official ergot, it still meets official standards. In addition, two-dimensional thin-layer chromatography of extracts of *Triticale* ergot against reference alkaloids resulted in the identification of the toxic alkaloids, ergonovine and ergocristine. These results point up the fact that ergoty *Triticale* grain has the potential of causing toxic manifestations when used as a feed grain.

Since the official grain standards of the United States (11) do not allow for more than 0.3% contamination with ergot, the extraction procedure was evaluated with *Triticale* grain artificially contaminated with 0.35% ergot so that it would be classified as ergoty. As seen from the results in Table 2, the method which we report in one case recovers 95.3% of the alkaloid from the grain contaminated with official ergot and 94.8% from the grain contaminated with *Triticale* ergot. We believe this to be the first report of a reliable method for the detection of ergoty grain after it has been ground.

In our attempts to isolate the alkaloids of ergot from ergot-contaminated grain, many different methods were tested. These methods included percolation with petroleum ether and then methanol, Soxhlet extraction with chloroform after moistening the grain mixture with methanol and NH_4OH, and finally, shaking on a rotary shaker with either 2% succinic acid solution or methanol. Official methods found in the British Pharmacopoeia 1948, the International Pharmacopoeia 1951, and the National Formulary XI and reported by Foster (9) for the determination of the water-soluble and water-insoluble alkaloids in ergot sclerotia, were also tested on the ergot-grain mixtures. All of these methods were found to be inefficient in the recovery of added alkaloid from ergot-grain mixtures, and therefore they are not reported in detail here. One of the major problems appeared to be the separation of the alkaloids from grain constituents which interfere with the colorimetric assay.

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