yield from the vacuum run on turkey mullein foliage is believed to be due mainly to slow diffusion of the volatiles within the foliage, at the low temperature of distillation, about 52 °C.

Relatively unstable compounds which appear to have been largely lost chemically during SDE at atmospheric pressure include *cis*-3-hexen-1-ol (peak 31) and its acetate (peak 27B) and two geometric isomers of 2,4-heptadienal (peaks 38B and 40). Benzaldehyde and *trans*-2-hexenal also were found at considerably higher concentrations in the vacuum-prepared extract than in the atmospheric-prepared extract. However, several aliphatic alcohols (peaks 15, 18, 30, and 46B), which would be expected to be stable, also were found at concentrations about 10 times higher in the vacuum-prepared extract. Perhaps these alcohols have higher permeation rates within the turkey mullein foliage and thus were extracted more fully than most of the other volatiles in the vacuum SDE run. An exception is 1-nonanol, which was about 100 times more concentrated in the vacuum-prepared extract. For this we have no explanation.

As related in the introduction, the essential oils from plants unpalatable to deer are inhibitory to deer rumen microbes. Oxygenated monoterpenes, notably the alcohols, have been implicated as the inhibitory components (Oh et al., 1968). The six monoterpenoid alcohols found in turkey mullein, in the atmospheric-prepared extract, add up to only 2.5%, a relatively low concentration compared to 14% in California bay and 56% in vinegar weed oil, both strongly inhibitory. The low monoterpenoid alcohol content of turkey mullein extract agrees with its reported moderate inhibitory effect (Longhurst et al., 1968). However, since it is unpalatable, it would be of interest to find the effect of the odor of other turkey mullein volatiles, in particular the cinnamate esters, on the behavior of deer.

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Analysis of Ergot Alkaloids in Flour

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A method has been developed for the analysis of flour for ergot alkaloids using liquid chromatography (LC) with fluorescence detection. Recoveries of ergometrine added to wheat flour at concentrations of 3.1 and 9.4 μg/kg were 66–72%, while recoveries of ergotamine, α-ergokryptine, ergocristine, ergosine, and ergocornine at levels of 14–16 and 41–48 μg/kg were 73–93%. Ergocristine (up to 62 μg/kg) was the major alkaloid detected in commercial wheat and rye flour, and LC patterns were similar to those of a sample of wheat ergot sclerotia.

Large-scale epidemics of human poisonings due to consumption of bread prepared from ergot-contaminated grain no longer occur. Strict grading standards, as applied in Canada and many other countries, do not permit grain containing ergot or more than a very small percentage of ergot to reach commercial food channels (Lorenz, 1979). Furthermore, cleaning and milling grain remove most of the ergot that might otherwise end up in flour (Shuey et al., 1973). Nevertheless, localized outbreaks of human ergotism may still happen due to negligence, as in Pont St. Esprit, France, in 1951 (Lorenz, 1979). Outbreaks of poisoning due to ergoty bajra (pearl millet) have been reported from the State of Maharashtra, India, during 1958, 1973, 1974, and 1975 (Bhat et al., 1976; Krishnamachari and Bhat, 1976). The alkaloids found in the bajra samples were clavine alkaloids and not alkaloids derived from lysergic acid and isolysergic acid. The method of analysis used by Krishnamachari and Bhat (1976) was capable of detecting 0.2 μg of total alkaloids/g using thin-layer chromatography (TLC) and colorimetry, but no quantitative results for individual alkaloids were given. Robbers et al. (1975) proposed a colorimetric assay for total ergot alkaloids in *Triticale* grain, but the lowest level tested was 7.6 μg/g, corresponding to 0.35% ergot in the grain. Colorimetric determination without separation has the

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disadvantage of including inactive alkaloids derived from isolysergic acid.

In view of the very low doses of some ergot alkaloids that give rise to toxic and pharmacological effects in animals and humans (Berde, 1978; Berde and Schild, 1978; Harrison, 1978; Lorenz, 1979) and the increased susceptibility of patients with certain diseases to their adverse effects, it is surprising that no attempt has been made previously to determine individual ergot alkaloids that might be present at low levels in foods derived from grains.

Methods used to separate and determine ergot alkaloids include TLC (Scott, 1980) and liquid chromatography (LC) (Dolinar, 1977; Szepesy et al., 1978; Yoshida et al., 1979). LC with fluorescence detection is a highly sensitive means of determining lysergic acid diethylamide (LSD), ergotamine, and other ergot alkaloids that possess a 9,10 double bond (Anderson et al., 1978; Christie et al., 1976; Heacock et al., 1973; Jane and Wheals, 1973; Johnson et al., 1977; Perchalski et al., 1975; Twitchett et al., 1978). Minimum detectable quantities of 0.005 ng for LSD (Johnson et al., 1977) and 1 ng for ergotamine (Anderson et al., 1978) have been reported. We have applied this technique to the analysis of ergot alkaloids in flour following simple extraction and cleanup by partition into dilute hydrochloric acid.

EXPERIMENTAL SECTION

Solvents. All solvents were purchased as glass distilled, except diethyl ether which in some experiments was of analytical reagent grade.

Extraction and Cleanup. Procedures were carried out in subdued light. Twenty-five grams of flour was twice extracted by shaking for 20 min with a mixture of 50 mL of methylene chloride, 25 mL of ethyl acetate, 5 mL of methanol, and 1 mL of 28% ammonium hydroxide solution. Larger quantities of flour (50 and 100 g) were extracted with proportionately more of the extraction solvent. The extracts were decanted and filtered under reduced pressure through a sintered glass funnel, which was then rinsed twice with 50 mL of methylene chloride. The combined extracts were evaporated to dryness by using a rotary evaporator with a bath temperature of ca. 30 °C. The residue was dissolved in a mixture of 35 mL of ether and 5 mL of methanol and extracted with two 60-mL portions of 0.5 N hydrochloric acid; the methanol was alternatively added after the second extraction as a preferred way of clearing emulsions. The combined acid layers were washed with 100 mL of hexane (which was discarded), made alkaline with 15 mL of 28% ammonium hydroxide solution, and reextracted with three 50-mL portions of methylene chloride. The combined organic extracts were evaporated under reduced pressure at ca. 30 °C, dissolved in 1 mL (or other suitable volume) of methanol, and filtered through a Millipore type AP25 prefilter and a 5-μm type LS Mitex disk.

Bread slices (454–680 g) were dried at room temperature and powdered in a Waring Blendor, and a 25–100-g subsample was extracted as described above.

Powdered wheat ergot sclerotia were extracted by soaking 100 mg in 5 mL of methanol for 2 h. Extracts were then carried through the cleanup procedure described for flour.

Isomerization of Ergot Alkaloids. Stock solutions containing approximately 500 μg/mL each of ergometrine, ergotamine, α-ergokryptine, ergocristine, ergosine, and ergocornine in methanol containing 0.1% acetic acid were refluxed for 2 h (Hofmann, 1964; Stoll, 1945).

Liquid Chromatography. Standard solutions of ergometrine (generally 0.1–0.025 μg/mL for fluorescence measurements) and ergotamine, α-ergokryptine (α-ergocryptine, Sigma Chemical Co.), ergocristine, ergosine, and ergocornine (all generally 1–0.2 μg/mL for fluorescence measurements) in methanol were stored in the freezer at −2 °C, preferably for not more than 1 week. A study was carried out on the stability of standard solutions stored under these conditions with the vials opened on the day of analysis (Table I). Ten to 25 μL of sample or standard (in methanol) was injected onto a 4.6 mm i.d. × 25 cm column packed with 5-μm Lichrosorb RP-8 (Brownlee Laboratories) using a Valco Universal Inlet with a 25-μL loop. The pump was an Altex Model 110. The flow rate for determinations was set at 1 mL/min; this was normally increased to 2 mL/min after elution of ergocristine. The flow rate for stability studies was 2 mL/min. The LC mobile phase was 43% (v/v) acetonitrile in a 200 mg/L solution of ammonium carbonate in distilled water (Dolinar, 1977) for measurement of ergosine, ergotamine, ergocornine, α-ergokryptine, and ergocristine in flour, and it was changed to 35% acetonitrile for ergosine and ergotamine in some of the flours and to 28% acetonitrile for ergometrine analysis. Acetonitrile was previously filtered through 0.5-μm Fluoropore type FH filters and ammonium carbonate solution through MF-Millipore type HA filters. Detection of alkaloids was by fluorescence measured with a Schoeffel FS-970 variable wavelength fluorometer at an excitation wavelength of 235 nm with a KV 370 emission filter. Detection by ultraviolet (UV) absorption was carried out by using a Waters Associates Model 440 absorbance detector with a 254-nm filter. Alkaloids were determined by measurement of fluorescence peak height and comparison with standard curves; the preferred attenuation was 0.05-μA full-scale. Corrections were made for moisture or solvent of crystallization present in the crystalline standards. After the samples were heated at 100 °C in vacuo, weight losses observed were 4.9, 2.5, 1.4, 0.0, 4.7, and 17% for ergosine, ergotamine, ergocornine, α-ergokryptine, ergocristine, and ergometrine. However, ergometrine contained 18.9% Cl, equivalent to 21.4% $CHCl_3$, and this value was used to allow for solvation. In addition, corrections were made to allow for the observed presence of 8.5% ergocornine and 1.3% α-ergokryptine in the ergocristine (undried).

Table I. Stability of Ergot Alkaloids in Methanol Solution

alkaloid	concn, μg/mL	% remaining[a] 10 days	14 days	21 days
ergometrine	0.049	92	85	79
ergotamine	0.365	90	92	71
ergocornine	0.49	96	97	73
ergokryptine	0.625	91	96	69
ergocristine	0.64	92	96	70

[a] Measured relative to freshly prepared standard solutions; means of duplicate determinations.

Recovery Experiments. Known amounts of ergot alkaloids in methanol were added to wheat flour (generally a 25-g sample) containing low background levels of the alkaloids and analyzed by the foregoing procedures using 43% acetonitrile (v/v) in aqueous ammonium carbonate except for determinations of ergometrine (28% acetonitrile). Analytical standards were prepared directly from the spiking solutions.

RESULTS AND DISCUSSION

Liquid chromatography (LC) with fluorescence detection is a highly sensitive means of detecting ergot alkaloids. We could readily detect as little as 0.5-ng amounts of ergosine,

Table II. Recoveries of Ergot Alkaloids Added to Wheat Flour

no. of replicates	concn of added alkaloid[b]	recoveries, %[a] ergometrine	ergotamine	α-ergokryptine	ergocristine	ergosine	ergocornine
5	w	70 ± 10[c]	88 ± 11	80 ± 7	86 ± 15		
2[d]	w	72	86	91	93		
1[e]	w	66	80	79	85		
2	w					78[f]	81
2	3w	66	90	85	89	74[f]	80

[a] Recoveries are corrected for contribution of blank, where applicable. [b] w = 3.14, 15.6, 16.0, 13.7, 15.2, and 15.8 μg/kg for ergometrine, ergotamine, α-ergokryptine, ergocristine, ergosine, and ergocornine, respectively. [c] Mean ± standard deviation. [d] Hexane wash omitted. [e] Analysis after 2.5 days. [f] 73% recoveries when determined in 35% acetonitrile solvent.

Table III. Estimated Concentrations (μg/kg) of Ergot Alkaloids in Flour[a]

sample	ergometrine, 28%[b]	ergosine 35%[b]	ergosine 43%[b]	ergotamine 35%[b]	ergotamine 43%[b]	ergocornine, 43%[b]	α-ergokryptine, 43%[b]	ergocristine, 43%[b]
wheat flour (blank)	0.27	ND[c]	ND	1.4	1.7	Tr[d,e]	Tr	2.7
wheat flour	0.41		0.69[e]		2.0	0.82[e]	1.1	3.5
wheat flour	0.40		0.69[e]		2.0	0.68[e]	0.88	3.6
wheat flour	0.75		1.5[e]		3.4	1.8[e]	1.7	7.7
wheat flour	0.75		1.5[e]		3.8	1.9[e]	2.2	8.2
wheat flour	1.7		2.0[e]		4.8	2.9[e]	2.7	10.5
whole wheat flour	2.0		3.1[e]		10.6	4.5[e]	4.6	21.8
whole wheat flour	2.3	10.3	3.5	11.2	17.1	5.3	6.5	26.6
whole wheat flour	2.8	7.2	3.1	5.3	12.4	3.7	5.8	16.8
graham flour	2.3	7.8	3.0	9.2	15.1	4.0	6.2	21.2
enriched flour	0.54	1.2	0.45	1.6	1.8	0.57	0.78	2.8
enriched flour	0.63	Tr	0.93	0.93	3.9	1.35	1.3	4.3
dark rye flour	10.4		10.8[e]		36.9	7.9[e]	10.3	62.2
dark rye flour	5.7	Tr	4.1	14	24	3.3	4.3	23.4
variability[f] estimate	8.7	14.2	8.6	18.1	6.9	9.1	7.7	6.1

[a] Means of duplicate determinations; those for ergotamine in 43% acetonitrile may include an interference. [b] % acetonitrile (v/v) in LC mobile phase. [c] Not detected. [d] Trace (<0.4 μg/kg). [e] Estimated by comparison with ergotamine standard and corrected for different response factors. [f] Average % differences of actual determinations from recorded means.

ergotamine, ergocornine, α-ergokryptine, and ergocristine and 0.1 ng of ergometrine. Standard curves were linear for all six alkaloids at the concentrations used. Other workers who have detected ergot alkaloids by LC with fluorescence (see beginning of paper) used excitation wavelengths ranging from 312 to 350 nm. With the FS-970 fluorescence detector, which has a deuterium lamp providing high energy in the short wavelength region, we found 235 nm to be the optimum excitation wavelength for sensitivity. Detection by UV absorbance at 254 nm required an attenuation of 0.005-μA full scale for use in the range 10–100 ng and in general was not suitable for flour analysis.

The method of extraction and cleanup developed for the analysis of flour resulted in good recoveries of added ergot alkaloids, which were primarily ergometrine, ergotamine, α-ergokryptine, and ergocristine (Table II). Standards of ergosine and ergocornine were obtained later, and fewer recovery studies were made. There were somewhat greater losses of ergometrine, which is more water soluble than the other alkaloids, but even so, recoveries averaged 70%.

The method was applied to the analysis of 14 rye and wheat flour samples (Table III), 8 of which were chromatographed until elution of the last alkaloid, ergocristinine, and the remainder until elution of ergocorninine. The most striking result is the qualitative similarity of the chromatographic patterns obtained from the extracts in the 43% acetonitrile system, both among themselves and in comparison with an extract of wheat ergot sclerotia (Figure 1). Extracts of wheat ergot shown in Figures 1, 2, and 3 were subjected to the same cleanup as the flour extracts and contained an extra peak (marked with an asterisk) not present in the crude ergot extract. The origin of this peak is unknown; a solvent blank was virtually free

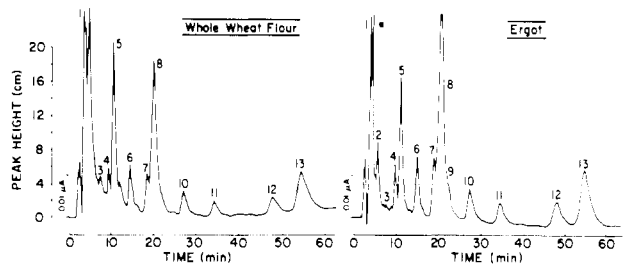

Figure 1. LC of extract of whole wheat flour (at left, 625-mg equivalent injected) estimated to contain 3.2 μg/kg of ergosine (peak 4), 18.1 μg/kg of ergotamine (peak 5), 6.1 μg/kg of ergocornine (peak 6), 6.2 μg/kg of α-ergokryptine (peak 7), and 25.8 μg/kg of ergocristine (peak 8) by fluorescence. Elution solvent was 43% (v/v) acetonitrile in aqueous ammonium carbonate (200 mg/L); flow rate was 1 mL/min; range setting was 0.05 μA full-scale At right, extract of wheat ergot (after acid cleanup, 25-μg equivalent injected) with fluorescence detection under the same conditions. Peaks 1, 2, 9, 10, 11, 12, and 13 are ergometrine, ergometrinine, ergosinine, ergotaminine, ergocorninine, α-ergokryptinine, and ergocristinine, respectively. Peak 3 is unidentified.

of interferences and the peak did not arise from decomposition of ergometrine during extraction and cleanup. Ergocristine was the major ergot alkaloid present in the flours. On the average, there appeared to be more ergotamine as estimated in the 43% acetonitrile system compared to the 35% acetonitrile solvent system (Table III), inidicating that there is an unresolved interference with the former system. Comparison of the chromatograms of the same whole wheat flour extract in the two systems illustrates this (Figures 1 and 2). A preliminary study on seven commercial wheat and rye bread samples indicated that a serious ergotamine interference is present in the

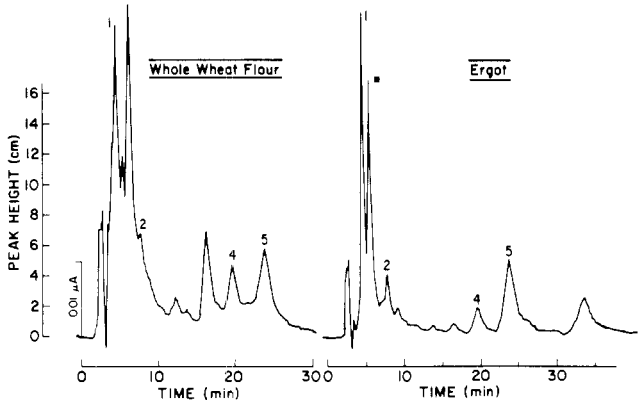

Figure 2. LC of extract of whole wheat flour (at left, 625 mg injected) containing an estimated 12.8 μg/kg of ergosine (peak 4) and 15.5 μg/kg of ergotamine (peak 5) measured by using 35% acetonitrile (v/v) in aqueous ammonium carbonate solution (200 mg/L). Other LC conditions were as in Figure 1. At right, extract of wheat ergot (after acid cleanup, 25 μg injected). Peaks 1 and 2 correspond to ergometrine and ergometrinine.

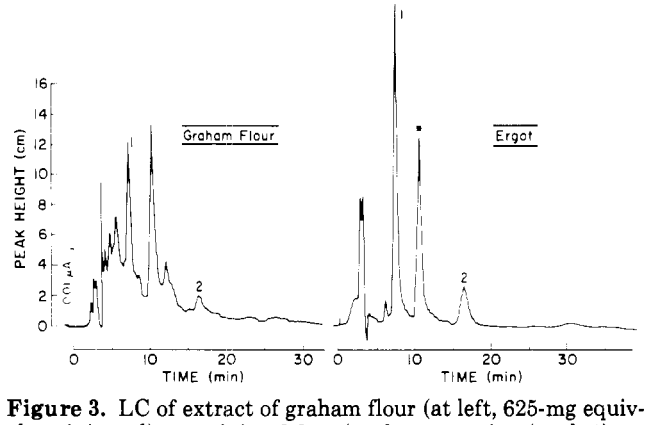

Figure 3. LC of extract of graham flour (at left, 625-mg equivalent injected) containing 2.3 μg/g of ergometrine (peak 1) estimated by fluorescence. At right, LC of wheat ergot (after acid cleanup, 25-μg equivalent injected). LC conditions were as in Figure 1, except elution solvent was 28% acetonitrile (v/v) in aqueous ammonium carbonate solution (200 mg/L). Peak 2 is ergometrinine.

43% acetonitrile system, with estimates (as ergotamine) ranging from 35 to 233 μg/kg; only two samples of rye bread contained ergotamine (2.4 and 11 μg/kg) when estimations were carried out in the 35% system. It is not clear which system is best for ergosine determination in flour. Ergometrine determination in graham flour is illustrated in Figure 3.

In conclusion, although improvements in methodology are needed in order to eliminate or resolve suspected interferences, it is apparent that low concentrations of ergot alkaloids are present in commercial flour. On the basis of the levels of total ergot alkaloids in the minimum therapeutic dose of prepared ergot (Wade and Reynolds, 1977), the concentrations we have found would not appear to constitute a health hazard for the general population. However, the toxicity of low doses of ergot alkaloids to humans should be further evaluated and an expanded survey of flour and grain foods for ergot alkaloids, together with a study of their stability during processing, should be undertaken.

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