pork ham butts used in the study may have been in the process of undergoing slight bacterial breakdown. This is indicated by the high polyamine content detected in several of the samples. It is, nevertheless, apparent that cooking pork in general decreases the concentration of amines. Putrefaction of pork, on the other hand, causes a significant increase in the concentration of certain amines, in particular spermine, spermidine, putrescine, and cadaverine. Commercial curing and smoking of hams cause no discernible effects on the concentration of the amine content (Table II).

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Received for review July 29, 1974. Accepted November 7, 1974.

Determination of Perloline by a Fluorometric Method

A fluorometric procedure for the determination of perloline, an alkaloid found in tall fescue (*Festuca arundinacea* Shreb.), has been developed. Interfering fluorescent compounds present in the 50% ethanolic extract of plant samples were removed by a cationic (H^+ form) chromatographic procedure. Perloline was found to give maximum fluorescence in 50–60% ethanol at a pH greater than 9.25. The maximum excitation and fluorescence wavelengths were found to be 450 and 512 nm, respectively, under these conditions. The overall recovery of this procedure was 92.5% with a high degree of precision. The detection limit of the fluorometric determination was better than 0.3 μg/ml.

Perloline, a fluorescent alkaloid in tall fescue (*Festuca arundinacea* Shreb.), has been implicated as one cause of poor performance of cattle grazing fescue during the summer (Bush *et al.*, 1970). To study the heritability of perloline content in genotypes of tall fescue, an improved method for the extraction and quantitation of perloline was needed. The first attempt to quantitate perloline (Bathurst *et al.*, 1943) was rapid although contamination in the chloroform fraction interfered with the colorimetric measurement (Jeffreys, 1964). A simple procedure developed by Gentry *et al.* (1969) lacked the precision desired for a heritability study. This paper describes a column chromatographic cleanup procedure and fluorometric determination of perloline in grass samples.

MATERIALS AND METHODS

Reagents used included: perloline monohydrochloride (Northern Regional Research Laboratory, Peoria, Ill.), tris(hydroxymethyl)aminomethane (Tris) buffer (Fisher Scientific Co., Fair Lawn, N.J.), and MSC-1 cation exchange resin (H^+ form) (Dow Chemical Co., Midland, Mich.).

Apparatus used included a Baird Atomic Fluorospectrometer Model SF-1.

Plant Tissue Samples. Samples were from a tall fescue breeding study located on the Bradford Farm, University of Missouri—Columbia. Samples were harvested Oct 24, 1973, and forced air dried at 55°. Dried samples were ground to pass a 1-mm screen, mixed, and stored in plastic bags at room temperature.

Extraction and Cleanup Procedure. To 125-ml erlenmeyer flasks were added 2-g samples of dried, ground plant tissue and 50 ml of 50% (v/v) ethanol–water (EtOH). The mixture was magnetically stirred for 2 hr. The plant residue was retained in a Büchner funnel fitted with Whatman No. 1 filter paper. The residue was washed with an additional 50 ml of 50% EtOH. The combined filtrate was passed through a column (14.5 mm i.d.) packed with 11–12 ml of cation resin at a flow rate of 2.5–3.5 ml/min. The column was washed successively with 20 ml of 50% EtOH, 30 ml of 95% EtOH, and 20 ml of deionized water. The major portion of cations was eluted with 50 ml of 7% aqueous NH_4OH (70 ml of concentrated NH_4OH diluted to 1 l. with H_2O) and discarded. If the eluate was still colored, additional 7% aqueous NH_4OH was used until the eluate became colorless. Perloline was eluted from the column with 60 ml of 14% NH_4OH in 95% EtOH (140 ml of concentrated NH_4OH diluted to 1 l. with 95% EtOH) into a flask. The ammonia was removed and the volume was reduced to 10–15 ml *in vacuo* at 40°. The perloline was transferred quantitatively into a 50-ml volumetric flask and brought to volume with 50% EtOH. Samples at this point could be stored in polyethylene bottles in a freezer until analyzed.

Analysis of Perloline. The above perloline samples were allowed to warm to room temperature. To dilute the samples to fit the range of the standard curve, an aliquot of each sample (1–10 ml), based on intensity of yellow color, was pipetted into a 25-ml volumetric flask containing 2.5 ml of 0.05 *M* Tris buffer (pH 10) and brought to volume with 50% EtOH.

Fluorescence of the samples was read on a fluorospec-

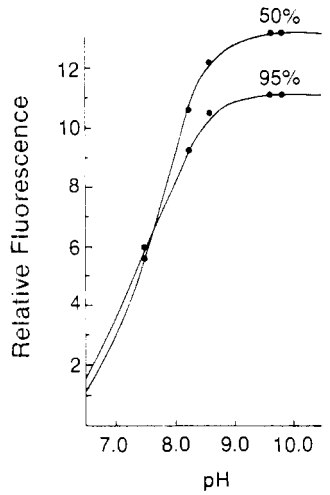

Figure 1. Effect of pH on relative fluorescence of perloline in 50 and 95% ethanolic solutions.

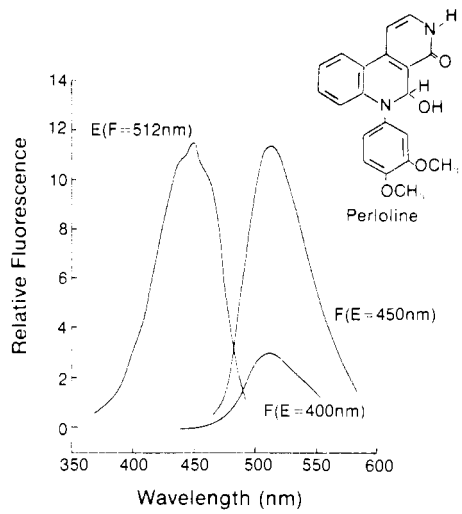

Figure 2. Excitation and fluorescence scans of perloline. Excitation scan $E(F = 512$ nm) with fluorescence set at 512 nm. Fluorescence scans $F(E = 400$ nm) and $F(E = 450$ nm).

trometer by setting the excitation wavelength at 450 nm and scanning the fluorescence from 509 to 515 nm (F_{max} = 512 nm).

Preparation of Standard Curve. To eight 25-ml volumetric flasks containing 2.5 ml of 0.05 *M* Tris buffer (pH 10) an appropriate aliquot of a standard perloline monohydrochloride solution (3 μg/ml) was placed in each flask to give a range of 0.36–2.04 μg/ml. Each standard was brought to volume with 50% EtOH. Fluorescence of the standards was read in the same manner as the samples. The standard curve was not linear above 0.84 μg/ml; therefore, concentrations had to be read from the standard curve.

RESULTS AND DISCUSSION

When first discovered perloline was found to fluorescece only in basic solutions (Grimmett and Melville, 1943). For this reason, in a preliminary experiment, the fluorescence of standard solutions containing 3 μg/ml of perloline was compared at different pH values. Tris buffer (0.05 *M*) and HCl were used to adjust the pH of the solutions. Simultaneously, a comparison of 50 and 95% EtOH as diluent was made. The results are shown in Figure 1. The fluorescence of perloline increased continuously from pH 6.5 to 8.5 and reached a plateau near pH 9.25 with both diluents. The fluorescence was stronger with the 50% EtOH diluent. In a subsequent experiment the concentration of ethanol was varied. The greatest fluorescence was achieved with 50–60% EtOH diluents. To assure maximum fluorescences in the samples, 0.05 *M* Tris buffer (pH 10.0) was used to adjust the pH and 50% EtOH was used as the diluent.

Fluorescence and excitation scans of a prepared standard were made to determine the fluorescent purity of the purified samples. Figure 2 shows the scans of a prepared standard. No change in spectra was noted after the standard had been passed through the cleanup procedure. Scans of the initial ethanolic plant sample filtrate were found to contain an interfering compound(s) which gave inaccurate results. Figure 3 shows scans of a grass sample filtrate before and after the chromatographic cleanup. The excitation scan revealed several peaks which were not present in that of the perloline scan. The fluorescence scan appeared normal when the excitation wavelength was set at 450 nm. If the excitation wavelength was set at 400 nm and a fluorescence scan made, the fluorescence maximum was shifted to 492 nm. This indicated the presence of a compound(s) other than perloline adding interfering fluorescence. The interfering compound(s) was completely removed by the column cleanup procedure. The values calculated from both scans are given in the caption of Figure 3. The interference caused a greater than 100% error in the value determined for that sample.

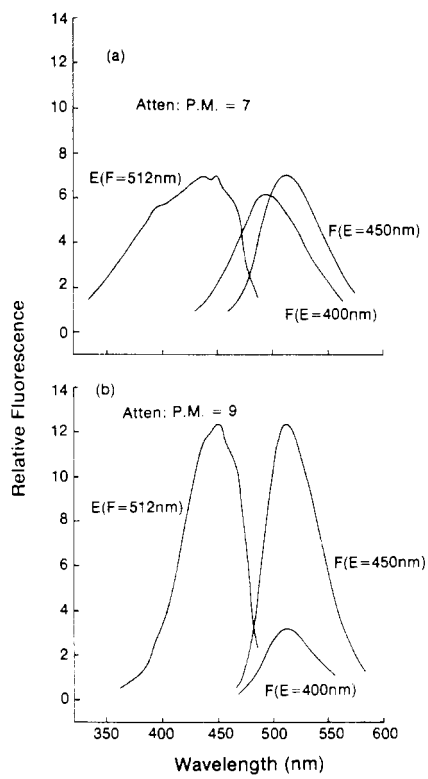

Figure 3. Excitation and fluorescence scans of a grass sample extract before (a) and after (b) chromatographic cleanup. Perloline per gram of dry matter calculated from the $F(E = 450$ nm) scans were (a) 0.16 mg and (b) 0.07 mg. Note difference in attenuation between a and b.

In an attempt to measure the fluorescence of a solution containing NH_4OH (eliminating the evaporation step) the samples were found to be unstable. A detectable change in fluorescence was evident during the time the samples were in the fluorometer. Both ethanol and ammonia concentrations in these solutions varied greatly after dilution and results were inconsistent. This observation stresses the importance of quantitative removal of the ammonia and volume reduction to assure a final concentration of 50–60% ethanol.

To determine the reliability of the overall procedure,

Table I. Reproducibility of Method Demonstrated by Independent Determinations

Sample no.	$\bar{X}$, mg/g of D.M.	% av dev. from mean	No. of det.
4-2	1.66	1.8	2
2-12	1.06	3.3	4
2-3	0.61	2.5	2
8-12	0.32	4.8	4
9-10	0.31	4.8	2
8-3	0.11	2.3	2
6-9	0.07	3.6	2
7-5	0.06	0.0	4

standard solutions of perloline monohydrochloride were used. Variation in recoveries between days and within runs was noted. By systematic elimination of possible sources of error which could account for this variation, only column flow rate remained to be checked. When the flow rate of the sample onto the cationic column was too slow (1.5 ml/min), there appeared to be more perloline trapped inside the matrix of the resin beads which then failed to be eluted in the final step. This resulted in low recoveries. When the flow rate was increased to 5.0 ml/min the perloline was distributed over more resin beads; however, eluting with alcoholic ammonia at this flow rate resulted in low recoveries. The low recovery may have been due to the lack of ammonium ion penetration for exchange in the matrix of the beads and/or incomplete solublization of the perloline which was eluted from the resin beads and precipitated on the column by the aqueous ammonia wash. By plotting recoveries for varied flow rates at each step, it was concluded that reproducible results could be obtained with a single flow rate for all steps in the cleanup procedure. A flow rate between 2.5 and 3.5 ml/min gave optimum recoveries. Using this range of flow rates, the amount of perloline recovered was a linear function of the concentration (0.1–3.0 mg/sample) applied to the cation column. By a graphical procedure, a slope factor of 0.925 was calculated. This corresponds to a 92.5% recovery. The same value was obtained when known amounts of perloline were added to grass samples at the time of extracting when compared to their base-line perloline values.

Reproducibility of the procedure was determined by independent analysis of several grass samples having different concentrations of perloline. Results of these experiments are presented in Table I. The procedure has proven to have a high degree of precision at all concentrations.

The procedure described is a sensitive, precise method for determining perloline in grass samples. In samples which do not contain interfering compounds the column cleanup could be by-passed and the loss of perloline reduced. The fluorometric method, with a detection limit in the range of 1.0 ng/ml, could be developed for different types of samples, such as feces or urine in ruminant metabolism studies.

ACKNOWLEDGMENT

The authors gratefully acknowledge the supply of perloline monohydrochloride furnished by S. G. Yates, Northern Regional Laboratory, USDA, Peoria, Ill., the supply of tall fescue genotype samples furnished by K. Asay, Agronomy Dept., University of Missouri—Columbia, and the technical assistance provided by L. Drake.

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Received for review May 28, 1974. Accepted December 4, 1974. Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 7025.

A Method for the Determination of Ethylenethiuram Monosulfide on Food Crops

A method was developed for the determination of ethylenethiuram monosulfide on food crops by gas–liquid chromatography. The procedure involves extraction with toluene followed by acid-base cleanup and yielded recoveries generally greater than 90%. The minimum detectable limit was 0.01 ppm.

Ethylenethiuram monosulfide (ETM) is a fungitoxic compound formed by aeration of ethylenebis(dithiocarbamate) fungicides (Ludwig *et al.*, 1954, 1955). The proposed structure of ETM (Thorn and Ludwig, 1954) has been revised (Pluijgers *et al.*, 1971) and the revision confirmed by ^{13}C nmr studies (Alvarez *et al.*, 1973) and by synthesis (Beer and Naylor, 1973).

While studies on the aeration of ethylenebis(dithiocarbamates) have indicated yields of up to 34% ETM (Ludwig *et al.*, 1954; Hylin, 1973), the levels of ETM remaining on crops as a result of agricultural usage remain uncertain. The following describes a glc method capable of determining ETM on food crops and supplementing the existing polarographic method (Engst and Schnaak, 1970).

EXPERIMENTAL SECTION

Materials. ETM was obtained from Robinson Brothers Ltd., West Bromwich, Staffs., England. The material was purified before use by column chromatography on silicic acid (Mallinckrodt, 100 mesh), using a developing solvent of chloroform–ethyl acetate (1:1) as described (Pluijgers *et al.*, 1971). The purified material had a mp of 125–126°.