known amount of spectro grade potassium bromide was added to the vial and mixed mechanically with the residue. The mixture was transferred to a $1^1/_2$ mm die, a pellet was pressed, and the spectrum of the pellet was recorded with an infrared spectrophotometer equipped with a 6 × beam condenser. A comparison of the spectrum with a known spectrum of rotenone (Fig. 1) showed that the compound isolated from the TLC strip was rotenone and illustrated that the technique was feasible for the isolation of a pure compound.

Work is being continued on separation and isolation of other rotenoid compounds.

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Paper chromatography of alkaloids of tall fescue hay

The alkaloids of tall fescue hay (*Festuca arundinacea* Schreb.) have been examined as a part of our work on the etiology of a cattle disease known as fescue foot[1]. Perloline has been isolated from tall fescue[2], and the presence of ergot-like alkaloids has been indicated by color tests[3]. However, no references have been found on the systematic separation of fescue alkaloids. This communication describes the separation of nine alkaloids by paper chromatography, and a method for isolating certain individual alkaloids.

Experimental

One to several hundred grams of toxic tall fescue hay were extracted by refluxing with 80 % aqueous ethanol (v/v). This extract was concentrated under reduced pressure to remove the alcohol, made basic with aqueous NaOH to pH 11, and exhaustively extracted with chloroform. The alkaloids were then extracted from the chloroform into *N*/10 HCl. The alkaloids were cycled again between chloroform and *N*/10 HCl. The final acidic solution was concentrated to a syrup at 40° under vacuum. Yield of crude alkaloids was about 0.1 % of the dry hay.

Whatman No. 1 paper* was washed with *N*/100 HCl and air dried at room temperature. Guidelines were drawn before washing so that development would be in the machine direction. Several hundred micrograms of crude alkaloid were placed on a spot, but if only single components were present, much smaller amounts were used (10–100 μg). Chromatograms were usually conditioned for 6–16 h with freshly prepared solvent (*n*-butanol–glacial acetic acid–water (10:1:3)) and developed by the descending procedure. The solvent front travels about 40 cm in 16 h at 25–27°. The developed chromatograms were air dried at 25–27°.

* Mention of trade names does not imply endorsement by the Department of Agriculture over similar materials not so named.

The dried chromatograms were scanned, first under white light and then under a long wave (3660 Å) ultraviolet lamp. The solvent front and any spots that fluoresced or absorbed were marked. The chromatograms were then sprayed with a color reagent to detect the remaining spots. Two types of color reagents were used, either a reagent which detects most alkaloids or reagents designed to form colors with specific functional groups.

Potassium iodoplatinate[4] was the best general alkaloid reagent examined and was ordinarily used. This reagent forms spots of different colors with the various alkaloids. The background color may be removed by washing the chromatograms with water after the reagent has dried. The spots of most alkaloids are not soluble in water. The limit of detection for fescue alkaloids is in the order of a few parts per million. Special reagents such as ninhydrin, ammoniacal silver nitrate, and diazotized *p*-nitroaniline[5] were used to detect reduced nitrogen groups, reducing compounds, and phenolic groups, respectively. Color development, except in the case of ninhydrin, was in air at room temperature.

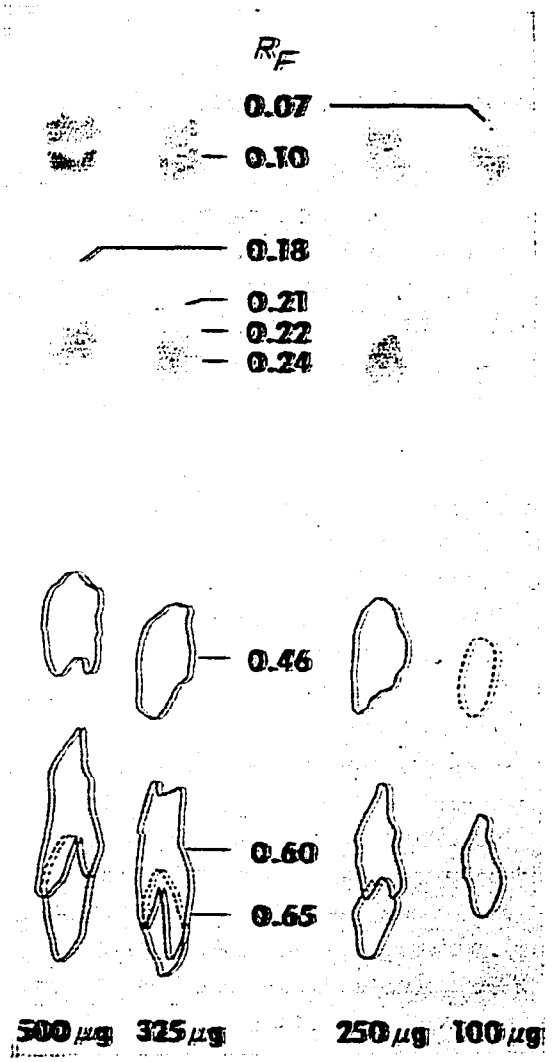

Fig. 1. Paper chromatogram of tall fescue alkaloids sprayed with potassium iodoplatinate. Conditions for development are given in the text.

For preparative paper chromatography, Whatman No. 3 MM paper was used without washing and was developed as described. Solutions were streaked across the chromatograms until the concentration was about 1 mg/cm. After the chromatograms were developed, areas containing particular alkaloids were cut out and eluted with $N/10$ HCl according to the method of DENT[6].

Results

There are at least nine different alkaloids in fescue (Fig. 1 and Table I). The major component is the alkaloid of R_F 0.10, representing approximately one-half of the

TABLE I

FESCUE ALKALOIDS DETECTED IN CRUDE EXTRACTS BY PAPER CHROMATOGRAPHY

R_F	*Color with potassium iodoplatinate*
0.07	Light gray
0.10	Blue-gray
0.18	Light gray
0.21	Light gray
0.22	Pink
0.24	Blue-gray
0.46	Yellow to tan*
0.60	No color**
0.65	Violet

* Yellow in white light.
** Spot fluoresces light blue in ultraviolet light.

alkaloidal material separated from tall fescue. Perloline appears at R_F 0.46. It was identified by its fluorescence, color, and ultraviolet spectrum[7,8].

The alkaloids of toxic tall fescue hay were compared with alkaloids in toxic fresh forage, non-toxic fescue hay, and rye grass. Alkaloids of the three fescue samples

TABLE II

R_F VALUES AND COLOR REACTIONS OF COMMERCIAL ALKALOIDS

Alkaloid	R_F	*Color with potassium iodoplatinate*
Caffeine	—	None
Theobromine	—	None
Theophylline	—	None
Ephedrine	—	None
Cacotheline	0.09	Tan
Brucine	0.43	Blue
Berberine	0.46	Tan
Pilocarpine	0.33	Gray
Quinine	0.52	Purple
Harmol	0.60	Light blue
Harmine	0.57	Violet
4-Hydroxy-L-proline	—	None
Ergotamine	0.77	None*

* Reacts if HCl fumes are present.

were chromatographically identical. The major alkaloids of rye grass were chromatographically identical to those in fescue. However, the alkaloid of R_F 0.65 was not detected, and there were small amounts of additional alkaloids in rye grass with an R_F value less than 0.07. When the alkaloid extract of a sample of fescue hay diseased with *Stemphyllium* sp. fungus was chromatographed, the alkaloid of R_F 0.10 and one or more of the alkaloids of R_F 0.18–0.24 were not present.

No similarities were found when fescue alkaloids were compared with a number of commercial alkaloids (Table II).

Chemical characterization of the major alkaloid, to be published elsewhere, might shed some light on the toxicity problem of tall fescue and provide the key to the structure of fescue alkaloids.

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