Analysis of Ergot Alkaloids in Endophyte-Infected Tall Fescue by Liquid Chromatography/Electrospray Ionization Mass Spectrometry

Richard A. Shelby*

Department of Plant Pathology, Auburn University, Alabama 36849

Jana Olsovska, Vladimir Havlicek, and Miroslav Flieger

Institute of Microbiology, Czech Academy of Sciences, Prague 4, Czech Republic

Previous investigators have identified a number of ergot alkaloids (EAs) in tall fescue (*Festuca arundinacea* Schreb.) infected by the endophytic fungus, *Neotyphoidium coenophialum* [(Morgan-Jones & W. Gams) Glenn, Bacon & Hanlin comb. nov.]. Their results, however, may have been confounded by the presence of the related parasitic ergot fungus (*Claviceps purpurea* [Fr.:Fr.] Tul.), which also produces EAs. Semipreparative HPLC was used to separate fractions giving a high fluorescence response in a sample of endophyte-infected (EI) tall fescue seeds, carefully examined to eliminate the possibility of *Claviceps* infection. Analytical high-performance liquid chromatography (HPLC) and LC/electrospray ionization mass spectrometry (ESI-MS) were used to identify EAs after isolation. Clearly identified in the spectra were ergine, ergovaline, ergosine, ergonine, and previously undescribed EAs, didehydroergovaline and aci-ergovaline, including their epimers. Several additional EAs may have been present, but structures could not be confirmed by their spectra. Not found in the isolated fractions were ergonovine, ergotamine, or lysergylmethylcarbinolamide. The developed HPLC method was used to determine the alkaloids in plants, culms, and seeds of EI tall fescue.

Keywords: *Neotyphodium coenophialum; Acremonium coenophialum; Festuca arundinacea, ergovaline*

INTRODUCTION

The endophyte of tall fescue, *Neotyphodium coenophialum* [(Morgan-Jones & W. Gams) Glenn, Bacon & Hanlin comb. nov.], infects millions of acres of tall fescue (*Festuca arundinacea* [Schreb.]) worldwide, causing economic losses in grazing livestock. Recent attention has been focused on the EAs being responsible, at least in part, for many of the animal toxicoses grouped generally under the term "fescue toxicosis". A detailed review of symptomology is beyond the scope of this paper; however, a good review can be found in Garner et al. (1993) and in Porter (1995). The early observations of the problem suggested that EAs might be involved, due to the similarity of the symptoms in those caused by the ergot fungi (*Claviceps* spp.), which also infect tall fescue and other pasture grasses. Isolation of the endophyte, and subsequent identification of its taxonomic affinity with *Claviceps*, made it clear that there were two species of fungi, either of which could cause similar symptoms in grazing livestock. This problem has confounded the isolation and identification of EAs from tall fescue, since the two fungi can simultaneously infect the grass host, and is further complicated by the fact that either fungus may escape superficial observation of seeds and plants.

The EA profile produced by *Claviceps purpurea* differs from that produced by the endophyte. Porter et al. (1987) used tandem mass spectrometry to identify ergotamine, ergosine, and ergocristine as the major EA components in sclerotia of *C. purpurea* on fescue, along with lesser amounts of six other EAs. They found this profile to be slightly different from *C. purpurea* growing on wheat and barley, mainly in higher levels of ergosine on fescue. The earliest observation of EAs identified from endophytes was from Porter et al. (1981), who identified ergovaline and several clavine alkaloids in cultures of the endophyte isolated from toxic tall fescue. Mass spectrometry had been used previously in the analysis of ergot cyclol alkaloids (Plattner et al., 1983), and later, Yates et al. (1985) used tandem mass spectrometry to confirm the presence of ergotamine, ergosine, ergonine, ergovaline, ergocristine, and ergocornine in endophyte-infected (EI) plant material. Yates and Powell (1988) published an HPLC method for EI fescue and identified several EAs as well as several fluorescent unknowns with this method. Their sample, however, contained trace amounts of sclerotia of *C. purpurea*, to which they attributed recovery of ergosine and ergotamine. Petroski and Powell (1991) identified a new alkaloid, ergine (lysergic acid amide), and ergonovine in tall fescue seeds. Identification was confirmed by MS, but the spectra were not published. TePaske and Powell (1993) measured levels of alkaloids in seeds and plants of 11 species from 5 genera of endophyte-infected grasses in which they found varying levels of ergine, ergovaline, ergosine, ergotamine, and ergokryptine.

From a quantitative standpoint, ergovaline appears to be one of the most abundant EAs of EI tall fescue (Yates et al., 1985; Yates and Powell, 1988; Porter, 1995) and so has received the greatest attention in terms of analytical methodology (Hill et al., 1993; Rottinghaus et al., 1991; Craig et al., 1994; TePaske et al., 1993; Shelby and Flieger, 1997). Generally, HPLC with fluorescence detection, which provides a combination of sensitivity, quantitative accuracy, and reproducibility

* Corresponding author. E-mail: rshelby@acesag.auburn.edu.

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necessary for routine laboratory analysis of this mycotoxin, was used. The essential methodology in these methods was utilized as the basis of initial isolation of EI fescue seed components by semipreparative HPLC, and also in the final separation by LC/ESI-MS.

The aim of this study was to determine and verify the structures of both known and unknown alkaloids in seeds, carefully examined for *C. purpurea* contamination, which produce a fluorescent signal, using LC/ESI-MS. In addition, the method was adapted for analytical use and applied to plants, culms, and seeds of EI fescue.

MATERIALS AND METHODS

Chemicals. Solvents used for extraction were ACS grade. Solvents used in HPLC were HPLC grade. The ergovaline standard was produced by F. T. Smith (Pharmacal Sciences, Auburn University). Ergosine and ergonine standards were provided by D. Romer and R. Geiger (Sandoz, Basel).

Extraction. Seed lot 7728 (Wilcox County, AL) was chosen from those submitted to our laboratory for routine endophyte analysis. This particular lot was selected because of high (100%) EI as determined by direct microscopic examination. Microscopic and macroscopic examination eliminated *C. purpurea* infection. The seed lot was ground dry to the consistency of a coarse flour, and 100 g was extracted with 500 mL of methanol/water (70:30) plus 1.5 mL of NH_4OH (pH 8.5), with gentle shaking for 8 h. After filtering, 300 mL of the filtrate was rotary evaporated at 40 °C for 1 h to a volume of approximately 100 mL. The pH was adjusted to 8.5 with NH_4OH, and the filtrate was extracted by partitioning with 3 × 50 mL of $CHCl_3$ in a separatory funnel. This was rotary evaporated to near dryness and dissolved in 2 mL of 70% alkaline methanol. Precipitates were removed by centrifugation and filtration through a 0.45 μm filter with methanol/water (70:30) plus 1.5 mL of NH_4OH (pH 8.5), with gentle shaking for 8 h. After filtering, 300 mL of filtrate was rotary evaporated at 40 °C for 1 h to a volume of approximately 100 mL. The pH was adjusted to 8.5 with NH_4OH, and the filtrate was extracted by partitioning with 3 × 50 mL of $CHCl_3$ in a separatory funnel. The combined $CHCl_3$ extracts were rotary evaporated to near dryness and dissolved in 2 mL of 70% alkaline methanol. Precipitates were removed by centrifugation and filtration through a 0.45 μm filter.

Partial Purification. Partial purification of individual ergot compounds from seed lot 7728 was accomplished using a semipreparative HPLC system: Waters 600E flow controller, Waters 610 pump with gradient valves, Waters U6K injector with a 2 mL loop, and Waters "Prep NovaPak" RCM 25 × 100 mm column. Detection was by fluorescence on a Waters 470 fluorimeter (excitation at 310 nm and emission at 415 nm) and simultaneously by UV on a Perkin-Elmer LC85B instrument at 225 nm. The mobile phase was methanol/water (30:70) plus 0.04% NH_4OH (A) and methanol/water (80:20) plus 0.04% NH_4OH (B). The flow rate was 5 mL/min in a linear gradient from A to B in 50 min, holding B for 10 min. One milliliter of the sample was injected into the system, and individual fluorescent fractions were collected from the HPLC outflow by fluorescence detector response. The detector voltage was monitored by observing the CRT display, and as response left the baseline, the outflow was collected by hand. Fractions were identified by retention time (Figure 1).

Analytical Method. A similar extraction procedure was used for other seed, culms, and grasses to follow the development of ergine and other analytes over the course of a growing season. Samples were collected from pastures of the Black Belt Substation (Marion Junction, AL), dried in a 50 °C forced-air oven, and ground to a fine powder. Samples (5 g) were extracted with 50 mL of methanol/water (70:30) plus 0.3% NH_4OH. After filtering, 25 mL of the filtrate was evaporated at room temperature overnight to a volume of approximately 10 mL. The pH was adjusted to 8.5 with NH_4OH, and the filtrate was extracted by partitioning with 3 × 5 mL of $CHCl_3$ in a centrifuge tube. This was evaporated at room temperature, and the residue was dissolved in 1 mL of 70% alkaline methanol. Precipitates were removed by centrifugation, and samples were injected directly into the HPLC apparatus. Chromatographic conditions were similar to those of the preparative method described above, except the analytical column was a Waters "NovaPak" C-18 (0.8 × 10 cm, particle size of 4 mm, RCM). The mobile phase was methanol/water (40:60) plus 0.03% NH_4OH (A) and methanol/water (80:20) plus 0.03% NH_4OH (B). The flow rate was 1 mL/min in a linear gradient from 100% A to 100% B in 45 min, holding 100% B for 10 min. Fluorescence detection was unchanged from the preparative method.

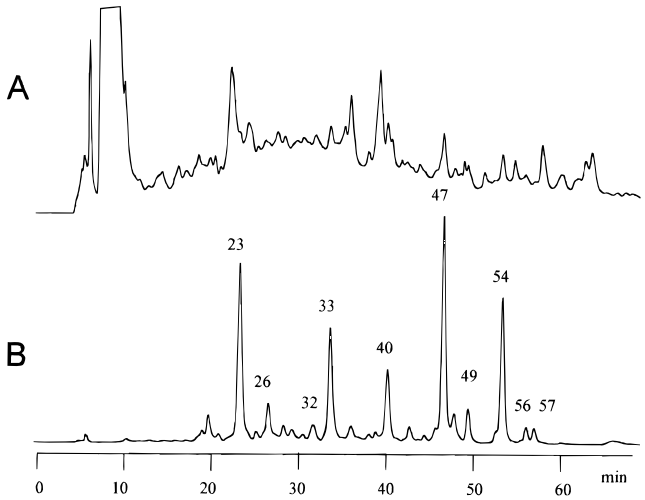

Figure 1. HPLC chromatograms of crude alkaloid extract of seed lot 7728: (A) UV (224 nm) and (B) with fluorescence excitation at 310 nm and emission at 415 nm. For the chromatographic conditions, see Materials and Methods.

LC/ESI-MS Analysis. Fractions collected from semipreparative HPLC were further analyzed on a reverse-phase column (Nucleosil 120-5, 250 × 2 mm inside diameter particle size of 5 mm). The mobile phase was methanol/water (90:10) plus 0.04% NH_4OH (A) and methanol/water (20:80) plus 0.04% NH_4OH (B). The flow rate was 150 mL/min in a linear gradient from 35 to 100% A in 60 min and holding 100% A for an additional 40 min. The column effluent was continuously infused through a stainless capillary held at 3.0 kV into the electrospray ion source. All positive ion electrospray ionization mass spectra were recorded on a double-sector Finnigan MAT 95 instrument (Finnigan MAT, Bremen, FRG) of BE geometry (magnetic sector preceding the electrostatic sector) equipped with the Finnigan ESI source. Polypropylene glycol ($M_r = 475$) (Merck) was used to calibrate the m/z scale of the mass spectrometer.

RESULTS AND DISCUSSION

Initial Separation. The initial semipreparative HPLC separation resulted in the isolation of five major and five secondary fractions (Figure 1B). The use of the fluorescence signal ensured that all of the fluorescent ergopeptine compounds were identified in the chromatogram. Simultaneous monitoring of the UV (Figure 1A) signal from these fractions suggested that the fluorescent fractions were not pure compounds, and some were estimated to contain as many as 10 UV-absorbing components. We also assumed that other components having no detector response were present in the sample, making LC/ESI-MS essential for detailed analysis. Tentative identification of several of these fractions was possible on the basis of comparison of analytical HPLC chromatograms with authentic standards. Additional evidence for identity was provided by formation of C(8) epimers of several fractions. After sitting at room temperature overnight, fraction 54,

Table 1. Liquid Chromatography−Electrospray Ionization Mass Spectra of Individual Compounds from Endophyte-Infected Tall Fescue Seed

fraction no.	scan no.	RT	$[M + H]^+$	ions observed	ID
23	118−130	9.8−10.8	?	237, 223, 221, 207, 205, 195, 181, 167	UNK 1
23	142−148	11.8−12.3	?	237, 221, 207, 205, 195, 181, 167	UNK 2
23	153−157	12.8−13.1	?	255, 237, 221, 208, 207, 195, 182, 181, 180, 167, 154	UNK 3
23 and 33	168−175	14.0−14.6	268	268 $[M + H]^+$, 223, 207, 221, 192, 181, 180, 167, 154	ergine
32	265−275	22.1−22.9	534	572 $[M + K]^+$, 556 $[M + Na]^+$, 534 $[M + H]^+$, 417, 235, 223, 191, 181, 167	aci-ergovaline
23 and 33	280−286	23.3−23.8	268	268 $[M + H]^+$, 224, 223, 207, 195, 180, 181, 167, 154, 153, 152	erginine
33	315−322	26.3−26.8	?	275, 253, 237, 235, 207, 195, 181, 167, 154	UNK 4
33	325−330	27.1−27.5	?	275, 253, 237, 235, 207, 195, 181, 167, 154	UNK 5
32	358−368	29.8−30.7	534	572 $[M + K]^+$, 534 $[M + H]^+$, 417, 235, 223, 191, 181, 167	aci-ergovalinine
40	444−460	37.0−38.3	532	570 $[M + K]^+$, 554 $[M + Na]^+$, 532 $[M + H]^+$, 514 $[M - H_2O]$, 486, 268, 223, 208, 180, 167	didehydroergovaline
40	498−508	41.5−42.3	532	570 $[M + K]^+$, 554 $[M + Na]^+$, 532 $[M + H]^+$, 514 $[M - H_2O]$, 486, 268, 223, 208, 180, 167	didehydroergovalinine
47 and 54	518−522	43.2−43.5	534	572 $[M + K]^+$, 556 $[M + Na]^+$, 534 $[M + H]^+$, 516$[M - H_2O]$, 488, 268, 223, 208, 180, 167	ergovaline
49 and 56	535−545	44.6−45.4	548	586 $[M + K]^+$, 548 $[M + H]^+$, 530 $[M - H_2O]$, 268, 208, 192, 180, 167	ergosine
47 and 54	572−584	47.7−48.7	534	572 $[M + K]^+$, 556 $[M + Na]^+$, 534 $[M + H]^+$, 516 $[M - H_2O]$, 488, 268, 223, 208, 180, 167	ergovalinine
49 and 57	586−588	48.8−49.0	548	586 $[M + K]^+$, 548 $[M + H]^+$, 530 $[M - H_2O]$, 268, 223, 208, 167, 142	ergonine
49 and 56	592−600	49.3−50.0	548	586 $[M + K]^+$, 548 $[M + H]^+$, 530 $[M - H_2O]$, 268, 223, 208, 167, 142	ergosinine
57	620−630	51.7−52.5	548	586 $[M + K]^+$, 548 $[M + H]^+$, 530 $[M - H_2O]$, 268, 223, 208, 167, 142	ergoninine

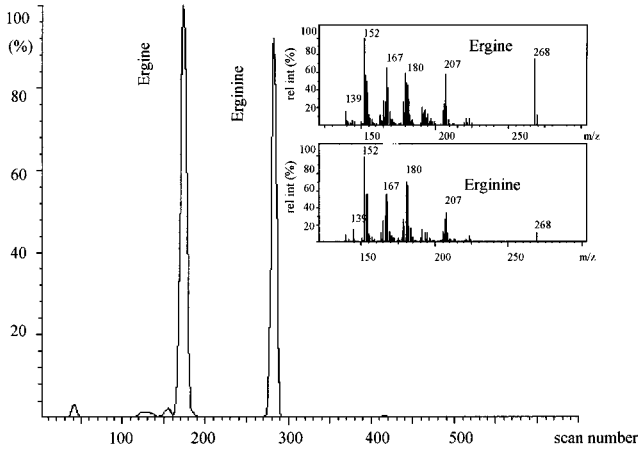

Figure 2. HPLC trace of ion *m*/*z* 152 of fraction 23. For the chromatographic conditions, see Materials and Methods.

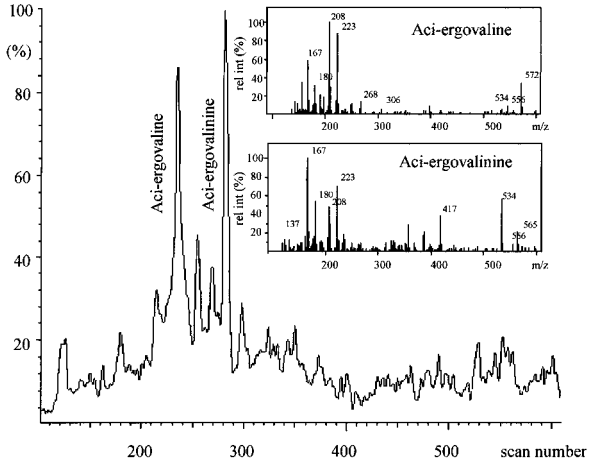

Figure 3. HPLC trace of ion *m*/*z* 208 of fraction 32. For the chromatographic conditions, see Materials and Methods.

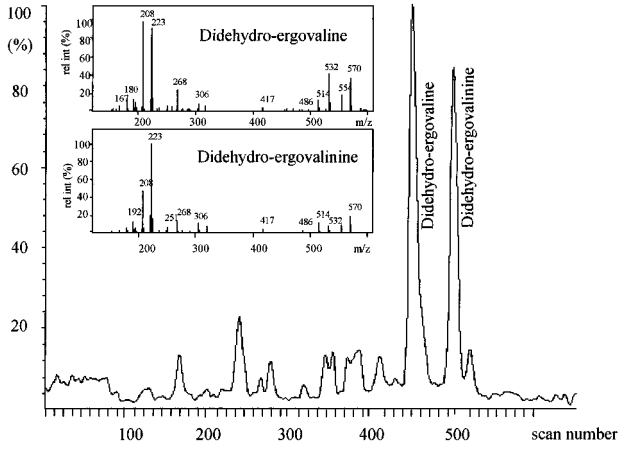

Figure 4. HPLC trace of ion *m*/*z* 208 of fraction 40. For the chromatographic conditions, see Materials and Methods.

tentatively identified as ergovalinine, spontaneously epimerized to produce ergovaline, and likewise, fraction 47, tentatively identified as ergovaline, epimerized to form ergovalinine. This phenomenon was also observed in fractions 23 and 33 (ergine and erginine), 49 and 56 (ergosine and ergosinine), and 49 and 57 (ergonine and ergoninine).

LC/ESI-MS. The corresponding electrospray ionization mass spectra of ergopeptines exhibit protonated molecules $[M + H]^+$ and cationized molecules, namely sodiated and potassiated, $[M + Na]^+$ and $[M + K]^+$ (Table 1; Figures 2−5). In order to obtain more detailed structural information, the electrospray ion source was adjusted with the heated capillary to 26 V, and the cone voltage was adjusted to 120 V. These conditions ensure the induction of fragmentation of the parent ions. Besides the elimination of one or two water molecules from the $[M + H]^+$ species, all generated ions are related to the ergine part of the molecule exclusively (Vokoun and Rehacek, 1975). Ion *m*/*z* 268 is the protonated lysergamide (Figures 2−5). The cation at *m*/*z* 223 describes the 9,10-unsaturated ABCD ergoline system (Figures 3−5). Ion *m*/*z* 208 is the most likely acylium-type ion of the elemental composition $C_{14}H_{10}NO$ (Figures 3−5). Ions *m*/*z* 192, 180, and 167 should be devoted to the ABC ergoline ring system. These fragments are present in most identified fractions (Table 1). The trace

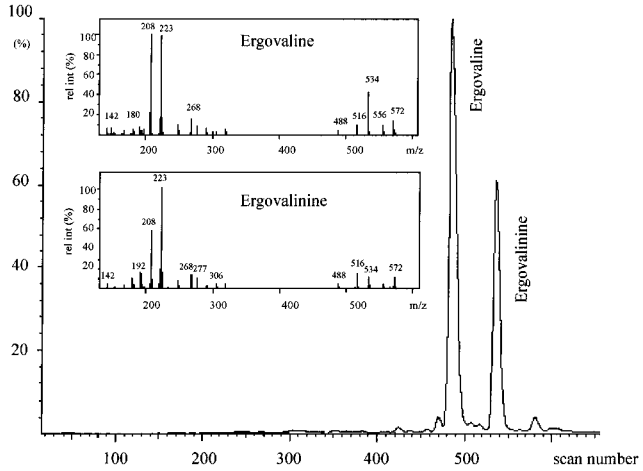

Figure 5. HPLC trace of ion *m/z* 208 of fraction 47. For the chromatographic conditions, see Materials and Methods.

of the ion *m/z* 208, which is the most prominent in corresponding mass spectra of ergopeptines (fractions 40−57, Table 1), was used for their detection on HPLC selected ion chromatograms. In all cases, these chromatograms reveal the doublet of C(8) epimers, which formed spontaneously in the interval from preparative isolation to LC-ESI/MS analysis. According to either retention data or relative intensities of selected fragment ions, the discrimination between epimers can be carried out. For example, the similar mutual intensity of fragment ions *m/z* 208 and 223 reveals the -ine isomer. On the contrary, the intensity of the *m/z* 208 lowered to 50%, when compared with that of the ion *m/z* 223, indicated the -inine isomer (i.e., ergovaline/ergovaline, Figure 5). This feature is common for all reported ergopeptines.

LC/ESI-MS analysis of fractions 23 and 33 gives the $[M + H]^+$ ion at *m/z* 268 which has been ascribed to ergine. Mass spectra of ergine and erginine (Figure 2) are nearly identical, and they cannot be distinguished by mass spectral fragmentation. In this case, the time profile of ion *m/z* 152 as the HPLC trace was chosen. Both fractions contain other compounds which give the fragmentation pattern characteristic of ergot alkaloids (Table 1). Unfortunately, the spectra of these compounds are contaminated with high amounts of impurities, so the identifications of the molecular masses of these compounds were not possible. According to known chromatographic behavior of ergot compounds, we can speculate on the structure of these unknown compounds. In our experience, the ergot alkaloid derivatives having a shorter retention time are lysergic and paspalic acids, compounds hydroxylated in position C(8), or glycosides of clavine alkaloids (Flieger et al., 1989, 1991, 1993; Havlicek et al., 1994). The 8-OH compounds could be a product of postproduction transformation by plant enzymes, for example, peroxidases. If these compounds are glycosides of clavine alkaloids, they are another possible source of animal intoxication from EI fescue. Ergot alkaloid glycosides, even present in minor concentrations, could be responsible for toxicosis because of their possible enhanced active transport through the blood−brain barrier; however, no toxicology studies on this new class of ergot compounds is available.

Fraction 32 gives, surprisingly, the same mass spectra as those in fractions 47 and 54, identified as ergovaline and its epimer (Figures 3 and 5). In the LC-ESI/MS, we found the only epimers of peptides giving low reversed-phase retardation are the aci-forms [C(2′)-

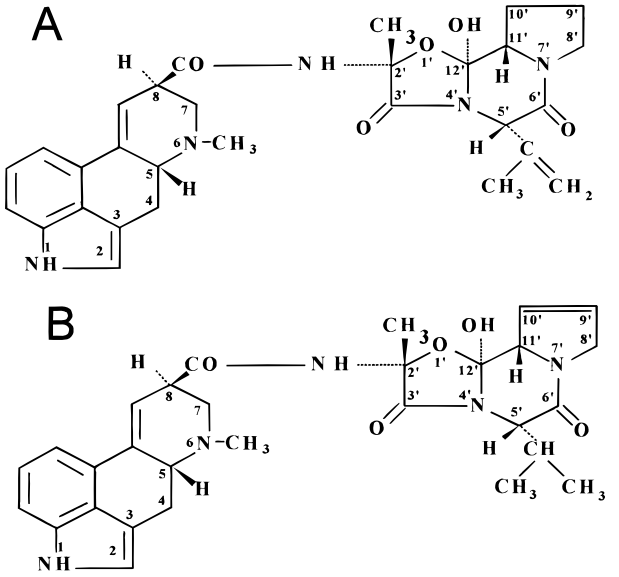

Figure 6. Proposed structures of didehydroergovaline.

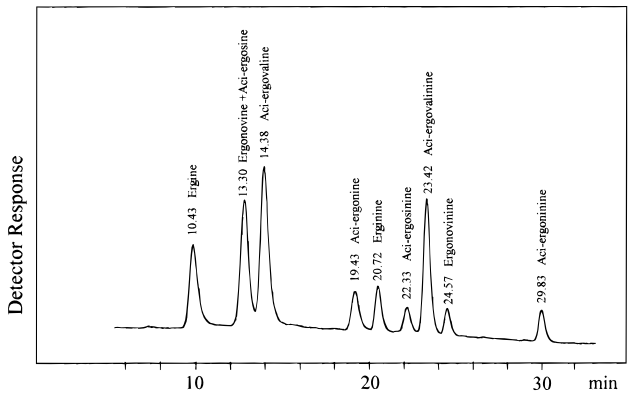

Figure 7. Detail from the HPLC chromatogram of standards of aci-ergovaline, aci-ergosine, and aci-ergonine, ergine, ergonovine, and their epimers. Chromatographic conditions were as follows: column, Waters "NovaPak" C-18, 0.8 × 10 cm, particle size of 4 mm RCM; mobile phase, 40:60 methanol/water + 0.03% NH_4OH (A) and 80:20 methanol/water + 0.03% NH_4OH (B). Flow rate of 1 mL/min in a linear gradient from 100% A to 100% B in 45 min, holding at 100% B for 10 min; fluorescence detector excitation at 310 nm and emission at 415 nm.

epimers] of ergot alkaloids. On the basis of these results, we epimerized an authentic standard of ergovaline under acidic conditions in order to obtain all four epimers [C(8) and C(2′)] (Rutschmann and Stadler, 1978). The resulting mixture was analyzed by HPLC, and the retention times of all epimers were determined. The same procedure was also used for ergonine and ergosine (Figure 7). On the basis of the retention data, fraction 32 was determined to be aci-ergovaline in the crude seed extract. Undoubtedly, aci-ergovaline is not an artifact of the extraction procedure, since no acidic conditions were used. We can only speculate on the source of its appearance. One possibility is the postproduction modification of ergovaline in the apoplast due to acidic conditions.

LC/ESI-MS of fraction 40 gave the same fragmentation pattern as that of ergovaline, except for the molecular ion, the $[M + Na]^+$ ion, and $[M + K]^+$ ion, all of which were 2 amu less than that for ergovaline (Table 1). This suggests an unsaturation within the cyclol moiety which we have tentatively identified as "didehydroergovaline" (Figure 6). Although somewhat speculative, unsaturation in the cyclol moiety may occur

Table 2. Quantitative HPLC Analysis of Seeds, Culms, and Plants of Endophyte-Infected Tall Fescue[a]

sample	ergine	ergovaline	aciergovaline	didehydroergovaline	ergosine + ergonine	total alkaloids
seed 1	2182.2	2276.2	930.4	444.3	404.5	6237.7
seed 2	1165.6	1058.4	1157.7	ND	309.8	3729.3
seed 3	3336.6	3588.2	1431.8	ND	679.0	9440.8
mean	2228.1	2307.6	1173.3	148.1	464.4	6469.2
culm 1	528.9	630.0	ND	ND	40.7	1125.1
culm 2	790.4	774.7	ND	ND	39.7	1405.9
culm 3	603.7	692.8	ND	ND	80.9	1307.0
culm 4	445.5	538.4	ND	ND	23.1	907.9
mean	592.1	659.0	ND	ND	48.1	1188.4
plant 1	230.2	232.6	ND	ND	15.3	505.8
plant 2	138.5	113.1	ND	ND	9.3	237.5
plant 3	268.1	207.3	ND	ND	25.2	496.7
plant 4	343.4	317.5	ND	ND	112.1	706.3
mean	245.1	217.6	ND	ND	48.9	495.0

[a] Nanograms of alkaloid per gram of dry weight of tissue based on integration of peak areas by Waters "Baseline" software, using standard curves generated from authentic standards.

naturally via the incorporation of dehydroergovaline during biosynthesis of the tricyclic peptide portion of the molecule (Figure 6A) or from a postproduction modification of ergovaline [i.e., hydroxylation of proline at position C(9′) followed by dehydration, resulting in the structure depicted in Figure 6B]. Another possibility would be the natural biosynthesis from the incorporation of hydroxyproline followed by dehydration, resulting in the structure in Figure 6B. Nevertheless, didehydroergovaline may contribute to the total alkaloid content of EI fescue, but the absolute structure and toxicology are the subjects of future investigations.

Fractions 47 and 54 gave expected spectra of ergovaline and its epimer (Figure 5; Table 1), whereas fractions 49, 56, and 57 gave spectra consistent with a mixture of ergosine and ergonine (i.e., fraction 49) and their epimers, ergosinine and ergoninine (fractions 56 and 57, respectively; cf. Table 1).

This analysis is important for the alkaloids which were not found. In particular, ergotamine, ergonovine, ergocristine, ergokryptine, and lysergylmethylcarbinolamide were not found in collected fractions. We believe that earlier observations of these alkaloids in EI fescue seeds resulted from *C. purpurea* infection, and not the *Neotyphodium* endophyte. Our own observations of these alkaloids in fescue seeds (data not shown) are usually associated with a low level of *C. purpurea* infection. Our future research will focus on the presence of *Claviceps* and *Neotyphodium* in seed samples submitted for routine diagnosis. Repeated analyses of seed by quantitative analytical HPLC may make it possible to more clearly define the two different EA spectra produced by these related genera of fungi.

Having established the relative retention times of several analytes of interest in our analytical system (Figure 7), we were able to determine their relative amounts in field-collected samples of EI tall fescue over the course of the fescue growing season in young plants (Figure 8A; Table 2), bolted culms (Figure 8B; Table 2), and finally seeds (Figure 8C; Table 2). As reported by Rottinghaus et al. (1991), we also observed an increase in ergovaline levels associated with flowering and seed formation (Table 2). We also detected a concomitant increase in the other analytes. Both aci-ergovaline and didehydroergovaline were detectable only in seeds, partly due to the presence of interfering peaks in plant tissue. The amounts of both ergine and ergovaline

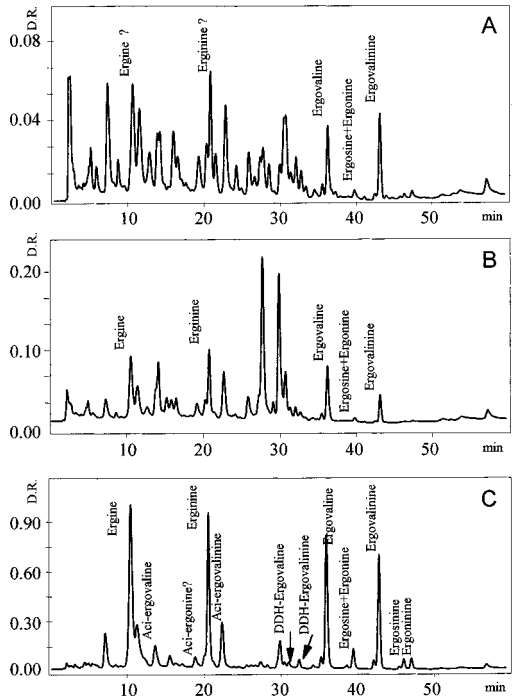

Figure 8. HPLC chromatograms of tall fescue. Chromatographic conditions were the same as those in Figure 7, except that NH_4OH was at 0.04%: (A) endophyte-infected plant, (B) endophyte-infected culms with inflorescence, and (C) endophyte-infected seeds.

increased correspondingly, and the ergine:ergovaline ratio remained relatively constant. If ergine were a postproduction product from the cleavage of peptide alkaloids, then its relative amount should increase with time. This did not appear to be the case. The analysis of plant material (Figure 8A) demonstrates that, even in the case of gradient analysis, the abundance of unknown fluorescent peaks makes the qualitative and quantitative interpretation of chromatograms questionable.

The presence of aci-ergovaline, didehydroergovaline, and ergine raises the possibility of postproduction biotransformations of ergot alkaloids in tall fescue as a product of grass−endophyte interaction. If this is the case, then other modifications of the ergot alkaloids may also be present, such as glycosylated alkaloids, as well as nonfluorescent clavines and other compounds which

could also contribute to the animal toxicoses. The grass host could harbor enzymes which transform the basic ergot structures into new, even more toxic compounds. The plant phloem could be an especially rich source of these enzymes and the aging, decomposing endophyte a likely source of parent compounds. This should be the focus of future research. Investigations of biotransformations in vitro, and by more specific extraction and purification methods, such as immunoaffinity columns, could help to explain the complicated toxicosis produced by the endophyte−grass associations.

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