Ergot Peptide Alkaloid Spectra of *Claviceps*-Infected Tall Fescue, Wheat, and Barley

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Clinical signs of tall fescue (*Festuca arundinacea*, Schreb.) toxicity in cattle, sheep, and horses may result from these animals ingesting ergot alkaloids contained in either the sclerotia of *Claviceps purpurea* parasitic on seed heads of tall fescue or grass parts and seeds of tall fescue infected with the endophyte *Sphacelia typhina* (*Acremonium coenophialum*). Tandem mass spectrometry was used to chemically separate and compare the ergot peptide alkaloids occurring in the sclerotium of *C. purpurea* parasitic on tall fescue with those found in this fungus parasitic on barley and wheat. The fescue sclerotia contained ergotamine (35.08%), ergocristine (30.65%), and ergosine(s) (26.96%) as the major peptide alkaloids. These were followed by minor percentages of ergokryptine(s) (2.95%), ergocornine (2.22%), ergostine (0.85%), ergovaline (0.30%), ergoptine(s) (0.18%), and ergonine (0.11%). Although the same ergot alkaloids were found in the sclerotia of *Claviceps*-infected wheat (minus ergonine) and barley, there were differences in alkaloid quantities. These differences are compared to the ergot peptide alkaloids reported earlier in foliage leaves of endophyte-infected tall fescue.

Toxicity studies provide evidence that all members of the family Clavicipitaceae are capable of producing ergot alkaloids and should be suspect in grass toxicity syndromes of cattle, sheep, and horses. In addition to *Claviceps*, endophytic fungi of *Balansia*, *Sphacelia typhina* (*Acremonium coenophialum* fide; Morgan-Jones and Gams, 1982), and possibly other species of endophytes have been shown to be the cause of cattle, sheep, and horse toxicity. Clinical signs include poor weight gains, abortions, and reproductive problems from various pasture grasses (Bacon et al., 1986; Mantle, 1969; Porter et al., 1979; Schmidt et al., 1982; Fletcher and Harvey, 1981; Wallner et al., 1983; Lyons et al., 1986; Thompson et al., 1986). The finding of the in situ production of ergot alkaloids in forage grasses by all these fungi suggests that information based on current methodology is needed to define the specific alkaloids produced by each group and species of fungus relative to its host. This information may be used to distinguish which organism is causing the toxic syndrome. Comparative data of the specific ergot alkaloids produced by *Claviceps* from forage grasses and cereal grains are lacking. We report on both the specific ergot peptide alkaloids and their relative percentages produced by *Claviceps purpurea* of tall fescue, compared to the ergot peptide alkaloids produced by the *C. purpurea* of barley and wheat. In addition, these data are compared to the previously reported ergot peptide alkaloids from endophyte-infected fescue.

MATERIALS AND METHODS

Fungus. Sclerotia of *C. purpurea* were hand picked from the inflorence of tall fescue (*Fustuca arundinacea* Schreb.) grown in outside garden plots located in Clarke County, GA. Hand-picked sclerotia of this fungus were collected from grain of wheat and barley growing in Idaho and were reported as toxic to cattle and swine (Krieger et al., 1983).

Extraction. Two-gram portions of each sclerotia were ground (mortar and pestle) and extracted (vigorous stirring, vortex mixer, 15 min each) with 5 × 20 mL of an acetone–2% tartaric acid (70:30, v/v) solution. The extracts were combined, filtered (Whatman No. 2), and evaporated to dryness (in vacuo) and the magma dissolved in 2% tartaric acid solution (30 mL). Total ergot alkaloids were analyzed in the crude extracts with *p*-(dimethylamino)benzaldehyde (PDAB) (Michelon and Kelleher, 1963). The tartaric acid solution (pH 2) was then extracted with 4 × 30 mL of CH_2Cl_2–*i*-PrOH (3:1, v/v); the extracts were combined, dried over anhydrous Na_2SO_4, filtered, concentrated (as above), and stored (N_2) for other investigations.

The aqueous acid solution was adjusted to pH 11 with NH_4OH and then extracted with 4 × 30 mL of CH_2Cl_2–*i*-PrOH (3:1, v/v); the extracts were combined, dried (anhydrous Na_2SO_4), filtered, concentrated to dryness (as above), and labeled the alkaloid fraction.

Identification. The residue of the alkaloid fraction was dissolved in 95% ethanol, and the individual alkaloids were separated by preparative thin-layer chromatography on 20 × 40 cm glass plates coated 0.75 mm thick with silica gel, GF-245 (Brinkman, Inc.). Identification was based on TLC (R_f) comparison with authentic standards (Porter et al., 1979, 1981) and by electron impact (EI) and chemical ionization (CI) mass spectroscopy of the individual alkaloids as previously defined (Porter et al., 1979, 1981; Porter and Betowski, 1981). Relative peptide alkaloid percentages were calculated from MS/MS data using the ratios of the signal for the major daughter fragment from each alkaloid as reported by Plattner et al. (1983). The response for all daughter fragments was assumed to be the same. The electron impact (70 eV) chemical ionization mass spectroscopy of the individual compounds isolated by preparative TLC was performed via direct insertion of probe samples on a Hewlett-Packard Model 5985B quadrupole mass spectrometer. MS/MS data were obtained on a Finnigan 4535/TSQ quadrupole mass spectrometer equipped with pulse positive–negative ion chemical ionization used in the positive and negative ion chemical ionization modes. Isobutane was used as reagent gas (0.2k Torr), and MS/MS analysis was conducted according to Plattner et al. (1983). All identified compounds corre-

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Table I. Relative Percent Ergopeptide Alkaloids in the Crude Alkaloid Fraction (Determined by MS/MS)

alkaloid	fescue	barley	wheat
ergotamine	35.80	48.96	9.59
ergosine and β-ergosine	26.96	3.77	1.97
ergonine	0.11	0.14	ND[a]
ergovaline	0.30	2.22	0.44
ergostine	0.85	0.39	0.74
ergoptine and β-ergotine	0.18	0.10	0.17
ergocornine	2.22	6.86	6.64
ergocristine	30.65	27.72	75.77
ergocryptine and β-ergocryptine	2.95	9.85	4.67
% total (PDAB)[b]	0.046 (0.46 mg/g)	0.092 (0.92 mg/g)	0.110 (1.10 mg/g)

[a] Not detected. [b] Cf. text and Michelon and Kelleher (1963).

sponded to authentic standards and literature: R_f, UV, M^+ (CI, EI) (Porter et al., 1974, 1979, 1981; Porter and Betowski, 1981; Plattner et al., 1983, and references cited therein).

RESULTS AND DISCUSSION

Using PDAB, analysis of the crude alkaloid fraction from the fescue ergot sclerotia revealed that the total ergot alkaloid content was 0.46 mg/g dry weight (0.046%) as compared to 1.1 mg/g dry weight (0.110%) and 0.92 mg/g dry weight (0.092%) found in wheat and barley, respectively (Table I). Ergotamine was the major peptide alkaloid identified (MS/MS) from the crude mixture of the sclerotia from fescue (35.8%) and barley (48.9%), while ergocristine (75.6%) was the major peptide alkaloid identified from the sclerotia of wheat. Ergocristine was quantitated at 30.65% and 27.72% in the crude matrix from the ergot sclerotia of fescue and wheat, respectively, while the content of ergotamine was determined to represent 9.59% of the extract from wheat. Ergosine(s) was the third most abundant ergopeptide alkaloid(s) in the fescue ergot (26.96%) while ergocornine was the third most abundant ergopeptide alkaloid found in barley and wheat (6.86% and 6.64%, respectively). This latter alkaloid represented 2.22% of the fescue alkaloids. Even though there were no comprehensive surveys of sclerotia in this study (i.e., individually or from other geographical locations), these findings correlated with those of others. Scott and Lawrence (1980) reported ergocristine as the major alkaloid in commercial wheat flour contaminated with sclerotia of *C. purpurea*. Riggs et al. (1968) presented no quantitative data but analyzed individual sclerotia and reported that 48% of all ergot alkaloid containing sclerotia from tall fescue produced ergotamine. Additional alkaloids reported by Riggs et al. (1968) from tall fescue and annual ryegrass–tall fescue hybrids included ergonovine, ergonovinine, ergocornine, and/or ergocristine and ergocristinine. We are able to verify the presence of ergonovine, ergonovinine, and chanoclavine (Porter et al., 1974, 1979) by TLC, EIMS, and CIMS in the alkaloid fraction of *Claviceps* sclerotia from fescue. Ergonovine to date has not been associated with endophyte-infected fescue. Bacon et al. (1979) and Porter et al. (1979) have shown that *Balansia epichloe* infected smutgrass (*Sporobolus poiretii*) produced ergonovine as its major alakloid, but the peptide alkaloids have not been isolated from the *Balansia* species. Since the primary focus of this study was the quantitative differences of the ergot peptide alkaloids, no attempt was made to define quantities of the other types of alkaloids (i.e., chanoclavine and ergonovine/inine). The occurrence of the ergosines and the ergovalines in ergot is generally not reported because of their low concentrations, and thier detection requires analytical methodology not readily available (Porter et al., 1981; Porter and Betowski, 1981). Thus, these two, as well as the ergoptines, have been only rarely reported from ergot sclerotia of rye, the ergot of commerce.

Young et al. (1981) have reported ergocristine followed by ergotamine to be the major peptide alkaloid isolated from Canadian rye ergot. Our report of ergovaline and ergosine in sclerotia from wheat and fescue is the first to our knowledge. The occurrence of these two rare groups of alkaloids, the ergovalines and the ergosines, has recently been identified as the major peptide alkaloid from endophyte-infected tall fescue leaves (Lyons et al., 1986; Yates et al., 1985) by the identical MS procedure (Plattner et al., 1983). These studies correlated with the in vitro production of ergovaline by the endophyte of fescue grown in saprophytic cultures (Porter et al., 1981, 1983). It has been established that ergot alkaloids produced within the sclerotia are not translocated to other parts of the grass (Riggs et al., 1968); they are however translocated from the sheath of endophyte-infected tall fescue to all grass parts (Lyons et al., 1986). Therefore, the presence of ergot alkaloids in leaf, stems, and seed would indicate that the grass is infected with the endophyte. *C. purpurea* being a localized ovarian infection of the grass seeds may be controlled by keeping the seed heads cut to prevent *C. purpurea* development. However, this control is not appropriate for endophytic infections where these compounds are translocated throughout the plant, i.e. that portion primarily used by grazing animals.

It appears that both the *C. purpurea* of tall fescue and the endophytic fungus produce a similar chemical spectrum of ergot alkaloids. It is not known whether the host, environment, and fungus strain are all equally important in the final biosynthesis of a specific ergot alkaloid by a particular strain of *C. purpurea*. There is a correlation between the available soil nitrogen and low level of soil phosphorus and the total alkaloid content during the parasitic production of ergot alkaloids on 15 different grasses (Kybal, 1963). However, in that study the same strain of fungus was not examined for a relationship between alkaloid production and the soil nitrogen–phosphorus content. Thus, the involvement of the genetics in a strain under different, controlled soil conditions is not known. Nevertheless, studies indicate that ergot alkaloids were produced regardless of the nutrient level of the soil, suggesting that soil fertility may not be important with this fungus (Kybal, 1963, 1964). Earlier studies of the *Balansia* species, another Clavicipitaceous fungus, indicated that the production of ergot alkaloids was grass host and fungus species specific (Bacon et al., 1981), while a study of endophyte-infected tall fescue indicated that the quantitative levels of ergot alkaloids were related to the nitrogen levles of the soil (Lyons et al., 1986).

The ergosines the ergovaline ratio found within sclerotia from infected barley and wheat, 1.7 and 4.5, respectively, examined in this study is in stark contrast to that of tall fescue, 89.9 (Table I). The ergosines have always been reported as minor alkaloids in sclerotia from cereals (Brunner, 1979), which is in agreement with our study. However, the relatively large percentage found in the sclerotia produced on tall fescue suggests that this fungus strain or sclerotia from this grass might serve as a source for these rare compounds that have only been used in limited toxicological studies due to their scarcity. This scarcity might not only reflect a specific fungus type, but also the difficulty in isolating and separating trace amounts

of these ergot alkaloids, and little attention is being given to procedures for their chemical identification and quantitation. Recent studies suggest that subclinical and combined toxicities of alkaloids may be a more insidious detriment to both livestock and human health than the individual compounds (Davis et al., 1983; Porter et al., 1983, 1985). Although extensive biological data exist on the ergot peptide and clavine alkaloids (Berde and Schield, 1978), little is known concerning their synergistic and/or subclinical toxicities as they exist in nature.

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Registry No. Ergotamine, 113-15-5; ergosine, 561-94-4; β-ergosine, 60192-59-8; ergonine, 29537-61-9; ergovaline, 2873-38-3; ergostine, 2854-38-8; ergoptine, 29475-05-6; β-ergoptine, 65756-55-0; ergocornine, 564-36-3; ergocristine, 511-08-0; ergocryptine, 511-09-1; β-ergocryptine, 20315-46-2.

LITERATURE CITED

Bacon, C. W.; Porter, J. K.; Robbins, J. D. *J. Gen. Microbiol.* **1979**, *113*, 119.
Bacon, C. W.; Porter, J. K.; Robbins, J. D. *Can. J. Bot.* **1981**, *59*, 2534.
Bacon, C. W.; Lyons, P. C.; Porter, J. K.; Robbins, J. D. *Agron. J.* **1986**, *78*, 106.
Berde, B.; Schield, H. O. In *Handbook of Experimental Pharmacology*; Springer-Verlag: New York, 1978; Vol. 49.
Brunner, R.; Stutz, P. L.; Tscherter, H.; Stadler, P. A. *Can. J. Chem.* **1979**, *57*, 1638.
Davis, C. B.; Camp, B. J. *Vet. Hum. Toxicol.* **1983**, *25*, 408.
Fletcher, L. R.; Harvey, I. C. *N. Z. Vet. J.* **1981**, *29*, 185.
Krieger, R. I.; Anderson, P. B.; Gallina, A. M.; Henningsen, G. M.; Naser, L. J.; Parnell, M. J.; Porter, J. K.; Stelle, E. E.; Warner, D. W. *Toxicologist* **1983**, *586*, 147.
Kybal, J. *Phytopathology* **1963**, *53*, 363.
Kybal, J. *Phytopathology* **1964**, *54*, 244.
Lyons, P. C.; Plattner, R. D.; Bacon, C. W. *Science (Washington, D.C.)* **1986**, *232*, 487.
Mantle, P. G. *J. Stored Prod. Res.* **1969**, *5*, 237.
Michelon, L. E.; Kelleher, W. J. *Lloydia* **1963**, *26*, 192.
Morgan-Jones, G.; Gams, W. *Mycotoxin* **1982**, *15*, 311.
Plattner, R. D.; Yates, S. G.; Porter, J. K. *J. Agric. Food Chem.* **1983**, *31*, 785.
Porter, J. K. Proceedings of the Tall Fescue Toxicoses Workshop, Atlanta, GA, March 17–18, 1983; pp 25–33.
Porter, J. K.; Betowski, D. *J. Agric. Food Chem.* **1981**, *29*, 650.
Porter, J. K.; Bacon, C. W.; Robbins, J. D. *J. Agric. Food Chem.* **1974**, *22*, 838.
Porter, J. K.; Bacon, C. W.; Robbins, J. D. *J. Nat. Prod.* **1979**, *42*, 309.
Porter, J. K.; Bacon, C. W.; Robbins, J. D.; Betowski, D. *J. Agric. Food Chem.* **1981**, *29*, 653.
Porter, J. K.; Robbins, J. D.; Norred, W. P.; Garner, G. C.; Bacon, C. W. *J. Agric. Food Chem.* **1985**, *33*, 34.
Riggs, R. K.; Henson, L.; Chapman, R. A. *Phytopathology* **1968**, *58*, 54.
Schmidt, S. P.; Hoveland, C. S.; Clark, E. M.; Davis, N. D.; Smith, H. A.; Grimes, H. W.; Holliman, J. L. *J. Anim. Sci.* **1982**, *55*, 1259.
Scott, P. M.; Lawrence, G. A. *J. Agric. Food Chem.* **1980**, *28*, 1258.
Thompson, F. N. *Theriogenology* **1986**, *25*, 575.
Wallner, B. M.; Booth, N. H.; Robbins, J. D.; Bacon, C. W.; Porter, J. K.; Kiser, T. E.; Wilson, R. W.; Johnson, B. *Am. J. Vet. Res.* **1983**, *44*, 1317.
Yates, S. G.; Plattner, R. D.; Garner, G. B. *J. Agric. Food Chem.* **1985**, *33*, 719.
Young, J. C. *J. Environ. Sci. Health* **1981**, *16*, 83.

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Indolealkylamines of *Desmanthus illinoensis* and Their Growth Inhibition Activity

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Methanol extracts of *Desmanthus illinoensis* roots inhibited the growth of tomato and radish seedling roots. Indole-3-aliphatic acids caused measurable root growth inhibition at 0.1 and 1.0 ppm, and the activity increased as the acid side chain decreased. Indole-2-carboxylic acid did not show significant activity. *N*-Hydroxy-*N*-methyl-1*H*-indole-3-ethanamine and 2-hydroxy-*N*-methyltryptamine were isolated and identified by ^{1}H NMR and MS/CI. Structural confirmation for these compounds was obtained with ammonia and deuteriated ammonia MS/CI.

Desmanthus illinoensis (Michaux), a legume frequently found growing in disturbed fields and roadsides in the southern part of the United States, was collected in Oktibbeha County, MS, and identified by the Botanical Institute at Mississippi State University. Previously, we reported the isolation, identification, and phytotoxic properties of the flavonoids from the leaves of this plant (Nicollier and Thompson, 1983). Nicollier et al. (1984) found phytotoxicity in the total root extracts of this plant.

MATERIALS AND METHODS

Extraction of *D. illinoensis*. Plant roots were washed and air-dried. The bark (378 g) was separated from the woody roots (350 g), and both were pulverized and extracted with 2.5 L of methanol. After filtering, the residue was refluxed in methanol for 2 h and filtered. Cold (room temperature, 24 h) and hot extractions were made of each filtrate. After TLC and HPLC showed no differences, the hot and cold extracts of bark and roots were combined to

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