Journal of Chromatography A, 652 (1993) 535–538
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 258

Isolation and quantification of ergovaline from *Festuca arundinacea* (tall fescue) infected with the fungus *Acremonium coenophialum* by high-performance capillary electrophoresis

Yinfa Ma*, Kevin G. Meyer and Dawood Afzal

Division of Science, Northeast Missouri State University, Kirksville, MO 63501 (USA)

Elizabeth A. Agena

Department of Chemistry, Simpson College, Indianola, IA 50125 (USA)

ABSTRACT

We developed an easy and sensitive high-performance capillary zone electrophoresis method for the determination of ergovaline in the seeds of *Festuca arundinacea* (tall fescue) infected with the endophytic, ascomycetous fungus *Acremonium coenophialum*. The seeds were extracted with chloroform, and the chloroform extract was filtered and evaporated with a Rotavapor. The concentrated extract was passed through a SM2 Bio-Beads and the alkaloids on the Bio-Beads were redissolved by methanol. A 60 cm × 75 μm I.D. fused-silica capillary was used for separation by capillary electrophoresis. A UV detector was used for detection, which was set at 250 nm. A 0.1 *M* sodium dihydrogenphosphate in 50% (v/v) methanol, pH 3.5, was used as a buffer. With this method, the ergovaline at low μg per kg seeds can be detected and quantified.

INTRODUCTION

Many grasses, including several agronomically important forage species, harbor endophytic, ascomycetous fungi. These fungal endosymbionts markedly modify the attributes of infected grass individuals, influencing the physiology, morphology, reproductive biology and palatability of grass hosts [1]. These effects are due to the ergopeptine alkaloids, especially ergovaline, produced by the endophytic fungus. The presence of high levels of ergovaline in endophyte-infected (*Acremonium coenophialum* Morgan-Jones and Gams) tall fescue is of interest because of its potential physiological activities [2–4], which typically exert central nervous system effects upon vertebrate consumers. Pharmacological studies [5] have shown that ergopeptine alkaloids, principally ergovaline, can interact with a variety of receptors and alter dopaminergic pathways [6]. Affected cattle gained mass poorly or lost mass, salivated profusely, had reduced milk production, and exhibited increased respiration rate [7,8] and body temperature [9]. Siegel *et al.* [9] has reported that approximately US$

* Corresponding author.

0021-9673/93/$06.00

$50 \cdot 10^6$ to $200 \cdot 10^6$ were lost to the cattle producers annually due to presence of higher level of ergovaline in the infected tall fescue. Therefore, developing a simple and fast method for analysis of ergopeptine alkaloids, especially ergovaline, will be very useful for agricultural development of the USA.

Tandem mass spectrometry (MS–MS) has been used for identifying the level of ergopeptine alkaloids in crude extracts of tall fescue [10]. However, the lack of MS–MS in many fescue research laboratories has hampered the analysis of ergopeptine alkaloids. Recently, high-performance liquid chromatographic (HPLC) methods for the analysis of ergovaline in fescue seed have been reported by several researchers [11,12]. Even though these methods can be used for quantitating ergovaline in endophyte-infected tall fescue, it was inadequate for fresh plant tissue analysis [11], and the complicated HPLC procedure limited the number of samples that could be analyzed conveniently per day.

High-performance capillary zone electrophoresis (HPCZE) has been proven to be a powerful technique in the separation of charged biomolecules with very high resolution [13,14]. The separation of standard ergot alkaloids by using cyclodextrins as a background electrolyte modifier in HPCE has been demonstrated by Fanali *et al.* [15]. However, the isolation and quantification of ergovaline in tall fescue, which is highly interested by American farmers, have not been studied by HPCE. In this paper, we developed an easy and sensitive HPCZE method for the quantitation of ergovaline in the endophyte-infected fescue seed. With this method, the ergovaline at low micrograms per kilogram of seed can be detected and quantified.

EXPERIMENTAL

Equipment

The HPCZE system (Model 3580) with a UV detector was purchased from ISCO (Lincoln, NE, USA). A positive high voltage was applied to the capillary by maintaining the injection end at a positive high potential while the cathodic end was held at ground potential. Data were collected with a Datajet computing integrator (Spectra-Physics, Mountain View, CA, USA). The capillary columns (Polymicro Techniques, Phoenix, AZ, USA) were 60 cm (35 cm to the detection system) × 150 μm O.D. × 75 μm I.D. The polymer coating was burned off 25 cm from the cathodic end of the capillary to form the detection window. The Rotavapor (Model RE111) was bought from Fisher Scientific (Fairlawn, NJ, USA).

Reagents and seed samples

All chemicals were of analytical-reagent grade unless stated otherwise. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). Ergovaline standard was a gift from Dr. George E. Rottinghaus, University of Missouri at Columbia. Ergonovine and ergotamine tartrate were purchased from Sigma (St. Louis, MO, USA).

HPLC-grade chloroform and methanol were purchased from Aldrich (Milwaukee, WI, USA). Sodium phosphate and hydrochloric acid were obtained from Fisher Scientific. SM-2 Bio-Beads were purchased from Bio-Rad (Richmond, CA, USA).

1991 Missouri endophyte-infected tall fescue seeds were obtained from Missouri Southern Seed Company (Rolla, MO, USA).

Pretreatment of the capillary column

All new capillary columns were filled with 0.01 *M* sodium hydroxide solution for about 30 min to clean the column. The column was then washed with deionized water and buffer solution. The capillary was ready for use thereafter.

Extraction and cleanup

A 1-kg amount of ground 1991 Missouri endophyte-infected tall fescue seed was mixed with 5 l of chloroform and stirred for 30 min. The liquid extract was separated from seed residue by filtering through cheesecloth, and the extract was roto-evaporated to 280 ml at a 30°C setting. The 280 ml of the extract were passed through Whatman 1 filter paper by vacuum filtration to remove any fine seed precipitant. A 56-g amount of SM-2 Bio-Beads was added to the extract and stirred for 1 h, so that the alkaloids would bind to the Bio-Beads. The liquid was removed by

vacuum filtration through Whatman 1 filter paper, and the beads were collected and washed 4 times with methanol (100 ml each time). The methanol eluent, which was concentrated to 25 ml by roto-evaporation, was ready to be analyzed by HPCZE.

HPCZE analysis

About 10 nl of methanol extract were injected for analysis. 0.1 *M* sodium dihydrogenphosphate in 50% (v/v) aqueous methanol, pH 3.5, was used as a buffer (the pH was adjusted by 1 *M* hydrochloric acid). HPCZE was operated at 30 kV for separation and 250 nm wavelength was used for UV detection.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of three standard alkaloids. Here it needs to be pointed out that peak B, ergovalinine, is the isomer of peak C, ergovaline, and they co-exist in the same standard solution. Ergovaline is very sensitive to light and heat. So, as time goes on, some ergovaline will be gradually isomerized to ergovalinine until the equilibrium is reached. Fig. 1 also demonstrate that femtomoles of alkaloids can be easily separated and detected. The calibration curve for ergovaline was linear over the range 100–1200 μg/kg, with a correlation coefficient 0.975. The detection limit was 90 μg/kg seed.

Fig. 2 shows the separation and detection of ergovaline in endophyte-infected tall fescue seeds. There was no interference from sample matrix. The unidentified peaks are due to the other compounds extracted from tall fescue seeds; the small peak eluting at 10 min was ergovalinine, the isomer of ergovaline, which was proven by the standard addition method in Fig. 3. From Fig. 2, we also can see that the ergotamine produced by the endophyte is too low to be detected, as was also found by the HPLC method developed by Rottinghaus *et al.* [11], in which they used ergotamine as an internal standard to analyze ergovaline. Based on the ergovaline peak areas of the sample seeds, the ergovaline contents in the 1991 Missouri endophyte-infected seeds were calculated to be 290 μg/kg seeds with a standard deviation of 16 for 5 analysis, which is compatible with the results analyzed by HPLC method [11]. This level is sufficient to induce symptoms of toxicity in heat-stressed cattle. The recovery of the ergovaline

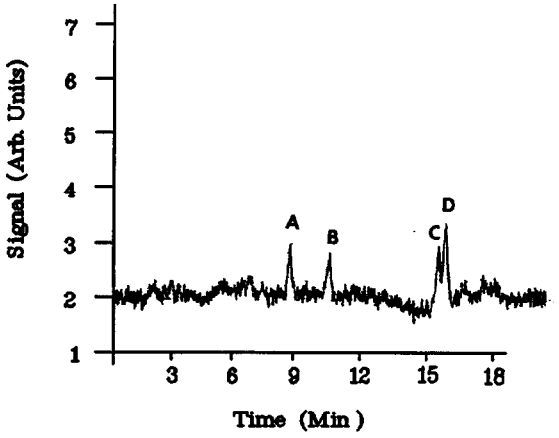

Fig. 1. The separation of three alkaloid standards which are commonly present in endophyte-infected tall fescue by HPCZE with a UV absorption detector. Sodium dihydrogenphosphate (1 *M*) in 5% (v/v) aqueous methanol, pH 3.5, was used as a buffer. Injection was made by a manual injector with a splitter. The injection volume was 10 nl. The concentration of the standards was 100 μM each. The electrophoresis was carried out at 30 kV on a 60 cm × 75 μm I.D. pretreated column. Detection wavelength was set at 250 nm. Peaks: A = ergonovine; B = ergovalinine (the isomer of ergovaline which co-exists in the ergovaline standard we obtained); C = ergovaline; D = ergotamine.

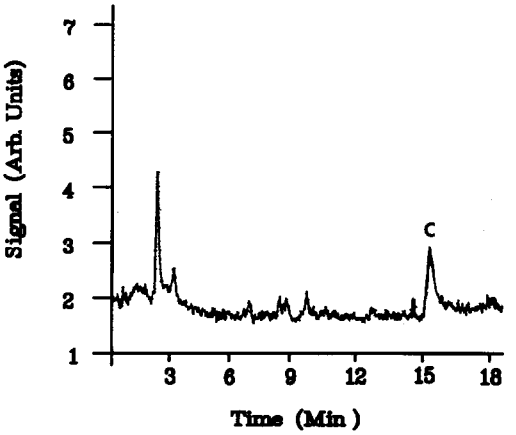

Fig. 2. The separation of ergovaline from endophyte-infected tall fescue seeds by HPCZE. Peak C = ergovaline. The electrophoresis conditions were the same as those of Fig. 1.

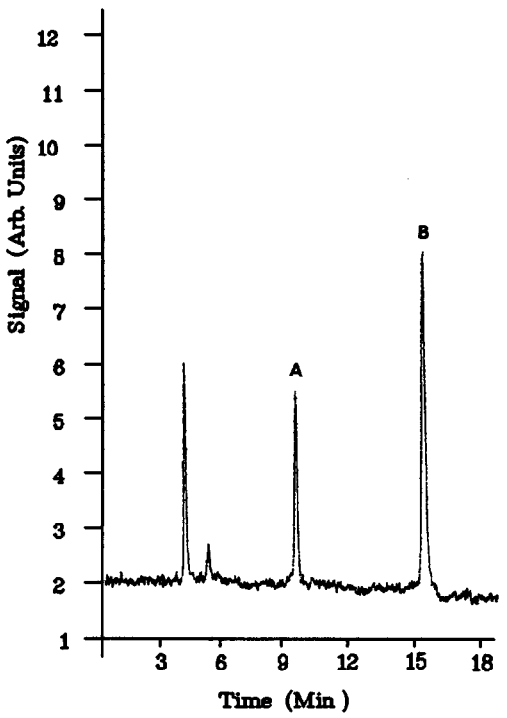

Fig. 3. The verification of ergovaline peak in Fig. 2 by standard addition method. Peaks: A = ergovalinine; B = ergovaline. The electrophoresis conditions were the same as those of Fig. 1.

has also been studied, and the average percent recovery is 95.1% for 5 extractions.

In order to verify the ergovaline peak in the sample electropherogram (Fig. 2), we also did a standard addition experiment, in which standard ergovaline was added to the methanol extract of the sample seeds. To 10 μl of methanol extract, 20 μl of 200 μl ergovaline standard were added and electrophoresis was carried out at the same conditions as those of Fig. 2. The electropherogram is shown in Fig. 3. From Fig. 3, we can see that the standard ergovaline peak exactly matches the ergovaline peak of the sample seed extract. This also proves that the small peak at 10 min was the ergovalinine peak.

CONCLUSIONS

We have developed an HPCZE method to analyze ergovaline in the endophyte-infected tall fescue seed. The method is easy, fast and sensitive, through which femtomoles of ergovaline can be detected. The method should be highly suitable for separation and determination of ergovaline in the endophyte-infected tall fescue seeds.

ACKNOWLEDGEMENTS

The authors sincerely thank Professor George E. Rottinghaus of the University of Missouri at Columbia for the ergovaline analytical standard and Dr. Ali S. Moubarak at the Department of Animal and Poultry Science, University of Arkansas, for the technical assistance on the extraction of ergovaline.

The work was supported by Faculty Research Grants awarded to Y.M. and D.A. from Northeast Missouri State University and NSF-REU grant BIO-9200423.

Y.M. and D.A. would like to thank the other participants of the NSF-REU grant at Northeast Missouri State University, especially Dr. Thomas Bultman, for the helpful discussions.

REFERENCES

1 K. Clay, *Ann. Rev. Ecol. Syst.*, 21 (1990) 275.
2 J.C. Read and B.J. Camp, *Agron. J.*, 78 (1986) 848.
3 K. Clay, *Ecology*, 69 (1988) 10.
4 C.P. West, E. Izekor, D.M. Oosterhuis and R.T. Robbins, *Plant Soil*, 112 (1988) 3.
5 B. Berde and E. Sturmer, in B. Berde and H.O. Schild (Editors), *Ergot Alkaloids and Related Compounds*, Springer, Berlin, 1978, pp. 1–28.
6 M.C. Henson, E.L. Piper and L.B. Daniels, *Domest. Animal Endocrinol.*, 4 (1987) 7.
7 R.J. Crawford, Jr., J.R. Forwood, R.L. Belyea and G.B. Garner, *J. Prod. Agric.*, 2 (1989) 147.
8 J.C. Read, C. Davis, B.J. Camp and E. Giroir, in *Proceedings, Forage and Grasslands Conference, Houston, TX*, American Forage and Grassland Council, Lexington, KY, 1984, pp. 240–243.
9 M.R. Siegel, M.C. Johnson, D.R. Varney, W.C. Nesmith, R.C. Buckner, L.P. Bush, P.B. Burrus, T.A. Jones and J.A. Boling. *Phytopathology*, 74 (1984) 932.
10 S.G. Yates, R.D. Plattner and G.B. Garner, *J. Agric. Food Chem.*, 33 (1985) 719.
11 G.E. Rottinghaus, G.B. Garner, C.N. Cornell and J.L Ellis, *J. Agric. Food Chem.*, 39 (1991) 112.
12 S.G. Yates and R.G. Powell, *J. Agric. Food Chem.*, 36 (1988) 337.
13 J.E. Wiktovowicz and J.C. Colburn, *Electrophoresis*, 11 (1990) 769.
14 M.V. Novotny, K.A. Cobb and J. Liu, *Electrophoresis*, 11 (1990) 735.
15 S. Fanali, M. Flieger, N. Steinerova and A. Nardi, *Electrophoresis*, 13 (1992) 39.