JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Purification of Fusaproliferin from Cultures of *Fusarium subglutinans* by Preparative High-Performance Liquid Chromatography

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Thirty-six *Fusarium* strains were grown on cracked yellow corn and evaluated for optimum fusaproliferin production, with *Fusarium subglutinans* E-1583 producing the highest levels (1600 μg/g). Three solvent systems were tested for extracting fusaproliferin from the cultures of *F. subglutinans* E-1583. Methanol gave the highest fusaproliferin recovery, followed by methanol/1% aqueous NaCl (55:45, v/v) and acetonitrile/methanol/H_2O (16:3:1, v/v/v). Hexane partitioning was effective in removing many impurities from the crude fusaproliferin extracts prior to the liquid chromatography step. Fusaproliferin samples were further purified by high-performance liquid chromatography (HPLC) with a C18 preparatory column using a mobile phase of acetonitrile/H_2O (80:20, v/v). The purity of the fusaproliferin was verified by analytical HPLC, GC/MS, ^{1}H NMR spectroscopy, and electrospray ionization (ESI) MS. The isolated fusaproliferin was shown to be free of impurities and can be used as a standard for routine analysis. Fusaproliferin was shown to be temperature-sensitive when samples were stored at room temperature (20−24 °C) for more than several days. After 30 days at 4 °C, approximately 8% of the fusaproliferin had been transformed to deacetyl-fusaproliferin; however, samples stored at −20 °C for 1 year contained only trace amounts of the deacetylated form.

KEYWORDS: Fusaproliferin; deacetyl-fusaproliferin; *Fusarium subglutinans*; mycotoxins

INTRODUCTION

Since the mid-1980s *Fusarium* mycotoxins, such as trichothecenes, zearalenone, fumonisins, and moniliformin, have received extensive attention because they are common contaminants in major cereal grains and their products. Tolerance guidelines have been set for fumonisins in foods and animal feeds in the United States (*1*), and some scientists have proposed advisory limits for deoxynivalenol, T-2 toxin, and zearalenone (*2*).

Fusaproliferin (**Figure 1**) was first isolated from cultures of *Fusarium proliferatum* ITEM-1494 and named proliferin (*3*), and it was subsequently further characterized and renamed fusaproliferin (*4*, *5*). The LC_{50} of fusaproliferin to brine shrimp, *Artemia salina*, is 53 μM (equivalent to 24 μg of toxin/mL), and its CC_{50} (cytotoxic concentration 50%) to the lepidopteran *Spodoptera frugiperda* cell line SF-9 is 70 μM and to the human nonneoplastic B-lymphocyte cell line IARC/LCL 171 it is 55 μM (*6*). In a toxicity test with chicken embryos, fusaproliferin was found to be the major cause for cephalic dichotomy, macrocephaly, and limb asymmetry when 1 or 5 mM pure fusaproliferin water/DMSO (38:62, v/v) solution was inoculated into the air sacs of the fertilized eggs (*7*).

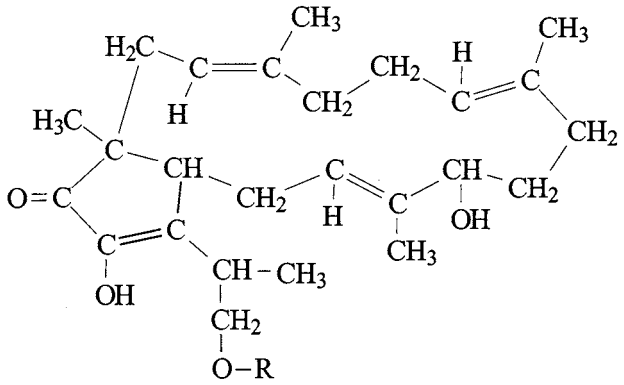

Figure 1. Chemical structure of fusaproliferin (FP) and deacetyl-fusaproliferin.

Strains from seven *Fusarium* species, *F. proliferatum*, *F. subglutinans*, *F. globosum*, *F. guttiforme*, *F. pseudocircinatum*, *F. pseudonygamai*, and *F. verticillioides*, can produce fusapro-

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10.1021/jf020904z CCC: $25.00
Published on Web 12/12/2002

liferin (*8*−*10*). Nearly all strains of *F. proliferatum* and *F. subglutinans* that have been tested produced fusaproliferin, and strains isolated from maize usually produce high levels of fusaproliferin (*6*, *8*, *11*). One of 4 *F. globosum* isolates and 3 of 29 tested strains of *F. verticillioides* produced low levels (10−35 μg/g) of fusaproliferin on maize (*10*, *12*). The highest fusaproliferin levels reported from cultures grown on cracked maize were 1725 μg/g by *F. proliferatum* (*12*) and 4309 μg/g by *F. subglutinans* (*13*). For the other three species, only the ex-type strains have been tested, and none of these strains produced high levels of fusaproliferin [*F. pseudocircinatum* (12 μg/g), *F. guttiforme* (85 μg/g), and *F. pseudonygamai* (130 μg/g)] (*9*). Strains of *F. proliferatum* and *F. subglutinans* appear to be the major sources of naturally occurring fusaproliferin in cereal grains.

Kostecki et al. (*14*) examined the effects of various substrates and temperatures on the production of moniliformin, fusaproliferin, and beauvericin by *F. subglutinans* ITEM-1434 and found that rye was the best substrate for the production of fusaproliferin. It has been reported that strains of *F. proliferatum* produce more fusaproliferin at relatively higher temperatures (25−30 °C) than at lower temperatures (<20 °C) and might be the main fusaproliferin producer on grains in tropical climates. *F. subglutinans* usually produces more fusaproliferin at relatively lower temperatures (15−20 °C) and forms little fusaproliferin at temperatures above 25 °C; thus, it is the primary fusaproliferin producer on grains in cooler climates (*12*, *14*).

Information on the incidence and contamination levels of fusaproliferin in foods and animal feeds is still very limited. Ritieni et al. (*15*) first reported natural contamination of fusaproliferin from visibly moldy maize in Italy, with one sample containing 500 μg of fusaproliferin/g. Munkvold et al. (*11*) reported the occurrence of fusaproliferin in animal feed outside Italy and found fusaproliferin levels in maize and feeds from Iowa at levels of 0.1−30 μg/g. Shephard et al. (*12*) found that the average fusaproliferin level in the positive samples of maize from subsistence farmers in South Africa was 33 μg/kg, with the highest level detected being 62 μg/kg. The highest fusaproliferin level found in maize from Slovakia with preharvest ear rot was 8 μg/g (*8*, *10*).

Randazzo et al. (*3*) described the only available procedure for recovering fusaproliferin from cultures of *F. proliferatum* grown on maize. This protocol is time-consuming and includes solvent extraction and partitioning, followed by a silicon column separation and a final TLC isolation. Our objectives in this study were (i) to select a *Fusarium* strain that produced high levels of fusaproliferin under laboratory conditions and (ii) to develop a simple and reliable HPLC method for the preparation of a fusaproliferin standard.

MATERIALS AND METHODS

Reagents. All of the solvents used were HPLC grade; the remaining chemicals were analytical grade. Acetonitrile, methanol, hexane, chloroform, and methylene chloride were purchased from Fisher Scientific.

A sample of fusaproliferin containing both the acetylated and deacetylated forms was provided courtesy of Antonio Logrieco (Istituto Tossine e Micotossine da Parassiti Vegetali del CNR, Viale Einaudi, Bari, Italy).

***Fusarium* Strains and Culture Media.** We evaluated 36 strains from *F. subglutinans*, *F. fujikuroi*, and *F. proliferatum* for fusaproliferin production after growth on yellow maize and selected *F. subglutinans* strain E-1583 for more detailed studies following that analysis.

Cracked yellow maize was collected from the feed processing facility at the Department of Animal Sciences and Industry, Kansas State University. Growth media were either Quaker Quick Grits (enriched white hominy, Quaker Oats Co., Chicago, IL) or cracked yellow maize kernels. Both maize kernels (900 g) and grits (680 g) were moistened and autoclaved twice at 121 °C for 1 h on consecutive days (*12*). Before the first autoclave cycle, 700 mL of deionized water was added to the cracked maize kernels and 350 mL to the grits. Before the second autoclave cycle, an additional 200 mL of deionized water was added to the maize and 250 mL to the grits.

E-1583 was grown on complete medium slants (*16*) at 25 °C for 5 days. Conidia were suspended in 1−2 mL of sterile 2.5% Tween 60 and used to inoculate two or three Petri dishes of autoclaved and cooled maize or grits (about 25−27 g). Inoculated Petri dishes were incubated at 24 ± 1 °C with a 12-h dark, 12-h light cycle. After 28 days of incubation, the cultures were removedfrom the incubator, dried in a forced-air chemical hood for 48 h at room temperature (20−25 °C), then ground to a fine meal with a food processor (Black & Decker, Inc., Shelton, CT), and kept at −20 °C until analyzed.

Recovery of Fusaproliferin Following Solvent Extraction and Hexane Partition. Three solvent systems, acetonitrile/methanol/H_2O (16:3:1, v/v/v), methanol/1% aqueous NaCl (55:45, v/v), and methanol (>99.9%), were used to extract fusaproliferin from the cultures of *Fusarium*. Samples (15 g) were extracted four times with each solvent on a wrist action shaker (Burrell Shaker model 75, Burrell Corp., Pittsburgh, PA) for 30 min each time (first with 45 mL and then 3 × 30 mL). Some of the crude extracts were first concentrated on a rotary evaporator at 40 °C to remove acetonitrile or methanol and then partitioned twice with 30 mL hexane. Most of the fusaproliferin was extracted into the hexane portion, with the water-soluble interfering substances remaining in the aqueous phase. The crude extracts or hexane portions from partition were evaporated to dryness on the rotary evaporator at 40−45 °C. The final residues were dissolved in methanol (4 mL), filtered through a 0.45-μm nylon filter, and stored in glass vials at −20 °C until further purification or analysis.

Purification of Fusaproliferin by Preparative Column HPLC. The fusaproliferin extracts were dissolved in methanol and purified on the same HPLC fitted with a 500-μL sample loop and a 250 mm × 10 mm, 5 μm, Alltima preparatory C18 reverse-phase column (Alltech, Deerfield, IL). The mobile phase was acetonitrile/H_2O (80:20, v/v) at a flow rate of 2.5 mL/min. The separation was monitored with a UV/visible diode-array detector set at 262 nm. HPLC retention times and UV absorbance profiles of the purified fusaproliferin were compared to those of the crude sample provided by Antonio Logrieco.

The HPLC-purified fusaproliferin solution was concentrated on a rotary evaporator at 45 °C. The concentrated fusaproliferin solutions were frozen overnight at −20 °C and then dried in a TDS-4A freeze-dryer (FTS Systems Inc., Stone Ridge, NY) for 48 h.

Analytical HPLC Evaluation of Extracts and Fractions Collected from the Preparative Column. The amounts of fusaproliferin recovered from the samples by the different solvents were evaluated with an HP 1090, Series II HPLC fitted with a 250 mm × 4.6 mm, 5 μm, Alltima analytical reverse-phase C18 column (Alltech Assoc., Deerfield, IL) and a UV/visible diode array detector (Agilent Technologies, Palo Alto, CA). The mobile phase was acetonitrile/water (80:20 v/v, 1 mL/min). The UV diode-array detector was set at 262 nm (*17*). Samples were dissolved in methanol or acetonitrile.

Ultraviolet Absorbance Measurements and Molar Absorptivity Determination. Freeze-dried fusaproliferin samples were weighed, dissolved in methanol, and diluted with methanol to give solutions containing 100, 50, 20, 10, and 5 μg of fusaproliferin/mL. The absorbance at 262 nm was measured with a Lambda 3B UV/visible spectrophotometer (Perkin-Elmer, Shelton, CT). We determined the molar absorptivity of fusaproliferin in methanol at 262 nm.

Gas Chromatography−Mass Spectrometry. Gas chromatography (GC) separations were performed with an HP 5890 GC fitted with an HP5970 MS selective detector (Agilent Technologies, Palo Alto, CA). The GC-MS data were collected and analyzed with HP5973 MSD Chem Station software and the Wiley7.0/NIST98 MS spectral library (Palisade, Newfield, NY). Samples were dissolved in methanol at a concentration of 300 μg/g. The samples were separated on a 15 m × 0.25 mm × 0.25 μm RTX-65 column (Restek, Bellefonte, PA) with a helium flow rate of 1 mL/min. The injector temperature was 270 °C, the source/

Table 1. Fusaproliferin (FP) Recovery[a] from 15 g of Culture Material by Different Solvents

sample	solvent	first and second extracts (μg)	third extract (μg)	fourth extract (μg)	total FP (mg)
grits	ACN/MeOH/H_2O	9090	1670	780	11.5
maize	ACN/MeOH/H_2O	15200	1990	730	17.9
	MeOH/1% NACL	17100	2580	1520	21.2
	MeOH	21000	2090	890	24.0

[a] Values are the averages of two replicates each; the differences between replicates were <3%.

transfer-line temperature was 280 °C, and the oven temperature was equilibrated at 200 °C for at least 2 min and then increased to 300 °C at a rate of 5 °C/min and held at that temperature for 20 min. The MS detector was operated in the EI mode at 70 eV, a mass range of 50−800, and a scan time of 0.5 s.

Electrospray Ionization (ESI) Mass Spectrometry. Electrospray ionization (ESI) mass spectrometry of the compounds collected from the HPLC preparations was evaluated with a TSQ Quantum triple quadrupole mass spectrometer (ThermoFinnigan Corp., San Jose, CA). Samples (100 pg/μL) for full-scan spectra were dissolved in 50% 2-propanol in water and infused into the ion source at a flow rate of 6 μL/min. ESI product-ion spectra were obtained with the same samples and conditions, except that 1.5 mTorr argon was used as the collision gas, and the collision voltage was set to 22 V.

NMR Analyses. Proton NMR analyses were performed with a Varian Unity Plus 400 NMR spectrometer (Varian, Palo Alto, CA). About 25 mg of freeze-dried, purified fusaproliferin was dissolved in $CDCl_3$ for proton NMR spectrometry (reference $CHCl_3$, $\delta = 7.27$).

RESULTS AND DISCUSSION

Selection of *Fusarium* Strain for Fusaproliferin Production. From the 36 *Fusarium* strains, we selected E-1583 because it produces high levels of fusaproliferin but relatively little beauvericin and no detectable moniliformin when cultured on cracked maize kernels. Strains that produce fusaproliferin usually also produce deacetyl-fusaproliferin (**Figure 1**) in a 3:1 ratio (*8*). The fusaproliferin/deacetyl-fusaproliferin ratio of strain E-1583 was approximately 5:1 when grown on cracked maize kernels and 9:1 when grown on grits.

Solvent Extraction and Hexane Partitioning Effects on Fusaproliferin Recovery. Fusaproliferin recovery ranged from 1600 μg/g with methanol extraction down to 1190 μg/g with the acetonitrile/methanol/H_2O combination (**Table 1**). The results also revealed that strain E-1583 produced more fusaproliferin on maize (1600 ppm) than on grits (766 ppm) under the conditions used. Background levels of fusaproliferin in the maize and grits used as growth media were not detectable (less than 10 μg/kg). The detection limit for fusaproliferin with our HPLC was 3.0 ng/injection. Extracts from the maize culture contained fewer impurities than did the grits culture.

In addition to the solvent, the length of the extraction process also affected the amount of fusaproliferin recovered. We extracted samples up to four times to get as much fusaproliferin from the cultures as possible. Because most of the reported fusaproliferin levels in contaminated maize were determined on the basis of one or two solvent extractions (*11*, *12*, *15*), the actual fusaproliferin levels in those samples might have been underestimated by as much as 20−40%.

The hexane-partitioned fusaproliferin samples are much cleaner than the nonpartitioned samples (**Figure 2**). As shown in **Figure 2**, the chromatograms of the hexane-partitioned sample (the bottom chromatograms) had much lower levels of interfering compounds close to the major fusaproliferin peak.

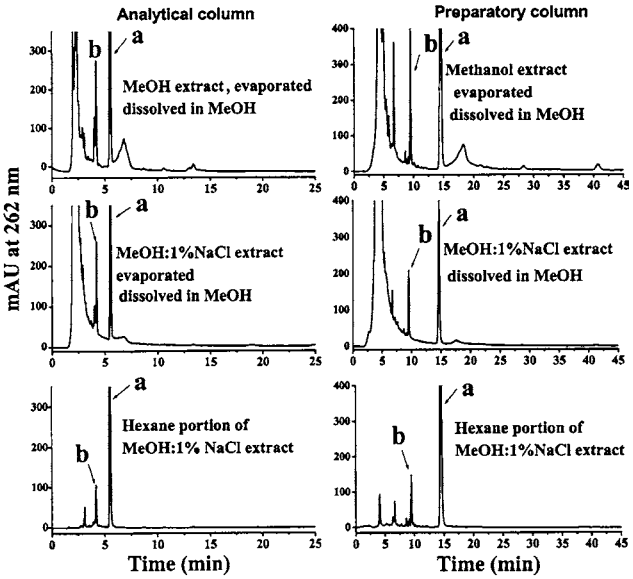

Figure 2. HPLC chromatograms of different fusaproliferin extracts on C18 columns with UV detection. The mobile phase was ACN/H_2O (80:20, v/v), and the flow rates were 1.0 mL/min for the analytical column and 2.5 mL/min for the preparatory column. (a) Fusaproliferin. (b) Deacetyl-fusaproliferin.

As a result, the hexane-partitioned samples gave better separation with a much larger injected sample. Approximately 80% of the fusaproliferin in the extract was recovered following the hexane partition process. Thus, hexane partition is an effective way to remove impurities from crude fusaproliferin extracts prior to HPLC.

Because fusaproliferin is not very water soluble, we had to wash the evaporating flask carefully with a small amount of methanol (~2 mL) and hexane used for partitioning after transferring the evaporator concentrated extracts to the separatory funnel and before the actual partition process. Otherwise, we lost up to 80% of the fusaproliferin on the inner walls of the evaporating flask. There are always some impurities in the samples well adsorbed onto the C18 preparatory column. When these impurities accumulate on the column, they decrease the separating efficiency of the column and might contaminate the purified fusaproliferin. To avoid this problem, the preparatory column should be reversely washed with acetonitrile/H_2O (90:10) after 5−10 injections depending on the amount of impurities in the samples.

Different solvents and sample preparation procedures might be suitable for different purposes. For detecting fusaproliferin levels in field samples, methanol extraction is the best choice because it gives the highest recovery and because the methanol extracts can be used directly for HPLC analysis, after being filtered, without the evaporating and hexane partitioning steps. For the preparation of the fusaproliferin standard, methanol/1% aqueous NaCl extraction followed by hexane partition is the best way to obtain a high-quality fusaproliferin standard from an HPLC C18 preparatory column because this solvent extracts lower levels of impurities than pure methanol and the acetonitrile/MeOH solvent, but still shows a reasonably high fusaproliferin recovery (**Table 1**).

Purity of the HPLC-Isolated Fusaproliferin Samples. When we used the published molar absorptivity ($\epsilon = 6000$ L/mol/cm) of fusaproliferin (*3*) to estimate the purity of our fusaproliferin isolates, the calculated purity was well over 100%. Obviously, this is not possible and might be due to a substantial amount of residual water in the original fusaproliferin used to

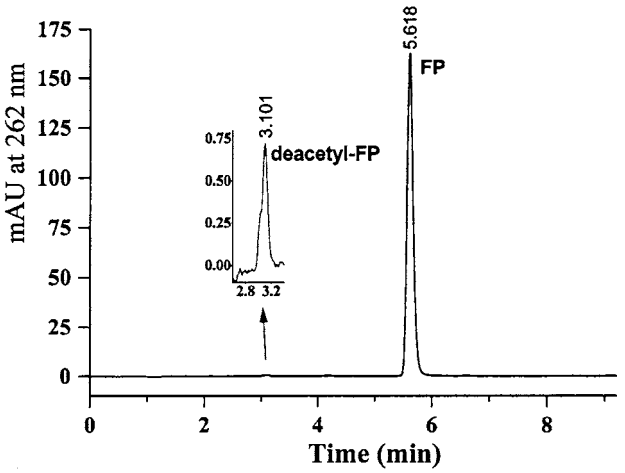

Figure 3. HPLC chromatogram of the purified fusaproliferin on a C18 analytical column. The inset shows the peak for deacetyl-FP at a retention time of 3.10 min. A trace amount of deacetyl-FP was detected in isolates concentrated by a rotary evaporator (0.15%).

determine the absorptivity (*3*). During our investigation, we noticed that fusaproliferin was hygroscopic and required extensive drying to remove the residual moisture. Our dried, purified fusaproliferin gave a molar absorptivity of 12 100 L/mol/cm (262 nm in methanol).

A typical analysis of the purified fusaproliferin by HPLC is shown in **Figure 3**. The only UV-absorbing material eluting from the column was the major fusaproliferin peak at 5.6 min. If samples were subjected to heat, such as through the use of a rotary evaporator to remove solvents, then we always detected traces of deacetyl-fusaproliferin by HPLC eluting at 3.1 min (**Figure 3**, inset). Both compounds had the typical UV absorbance spectra of fusaproliferin and were identical to the reference material. The presence of a small amount of the deacetyl form is not unexpected because of the labile nature of the acetate group (**Figure 1**).

The GC data were in agreement with the HPLC evaluations. GC analyses of the two major forms of fusaproliferin are presented in **Figure 4**. **Figure 4A** and **B** shows the analysis of purified fusaproliferin and deacetyl-fusaproliferin separately, whereas **Figure 4C** shows the separation of a mixture of fusaproliferin and deacetyl-fusaproliferin. It is apparent that separation of the two forms of fusaproliferin is easily achieved with the RTX-65 column, and thus we can conclude, along with the HPLC data, that there is little if any of the deacetyl-fusaproliferin in the purified material.

Supporting mass spectral data are presented in **Figure 5**. The peak eluting at about 19.2 min has a mass spectrum very similar to that reported by Ritieni et al. (*4*) and has a characteristic molecular ion at *m*/*z* 444, an $M^+ - 18$ fragment at *m*/*z* 426, and a base peak at *m*/*z* 81 (**Figure 5A**). The mass spectrum of the deacetyl-fusaproliferin eluting at 19.8 min (**Figure 5B**) has a product-ion fragmentation pattern similar to that of the intact fusaproliferin except for the molecular ion at *m*/*z* 402 and an $M^+ - 24$ ion at *m*/*z* 378. Although we did not detect any other peaks in the purified fusaproliferin sample, we did see several peaks eluting from 14 to 17 min in the deacetyl-fusaproliferin. These peaks probably represent minor thermally induced breakdown products resulting from the high temperatures of the GC runs. We were not able to match these compounds with any listings in the Wiley/NIST98 MS spectral libraries.

To further evaluate the purity of our material, we used electrospray ionization (ESI) in both the positive- and negative-

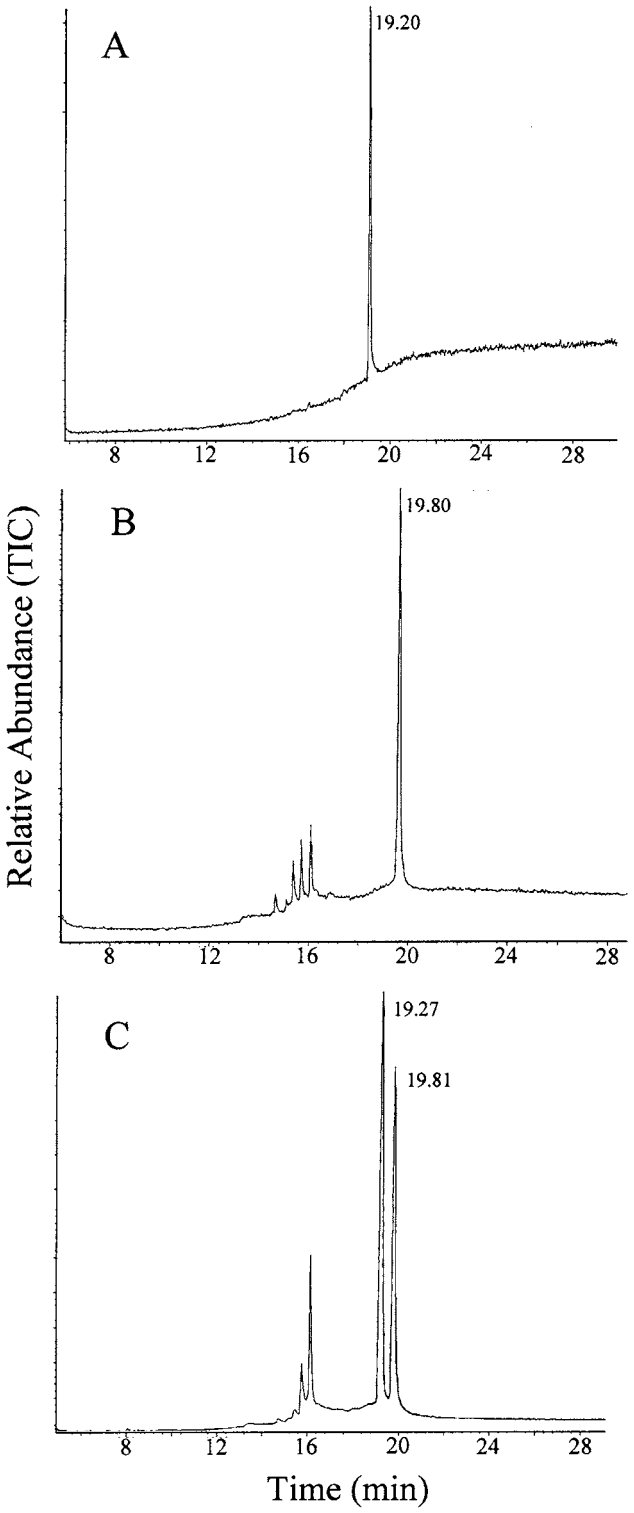

Figure 4. GC chromatograms of fusaproliferins on a RTX-65 column. (A) Purified fusaproliferin. (B) Purified deacetyl fusaproliferin. (C) Mixture of purified fusaproliferin and deacetyl-fusaproliferin.

ion modes. Direct flow analysis was used so as to minimize interferences from solvents typical used in HPLC. **Figure 6A** shows an electrospray mass spectrum of the purified fusaproliferin in the positive-ion mode. The spectrum show an $(M + H)^+$ ion at *m*/*z* 445.5, an $(M + H - 18)^+$ ion at *m*/*z* 427.4 and an $(M + H - 32)^+$ ion at *m*/*z* 409.5. These ions are similar to those reported by Sewram et al. (*18*), who also evaluated fusaproliferin with positive-ion electrospray.

In the negative ESI mode, the major fragments were found

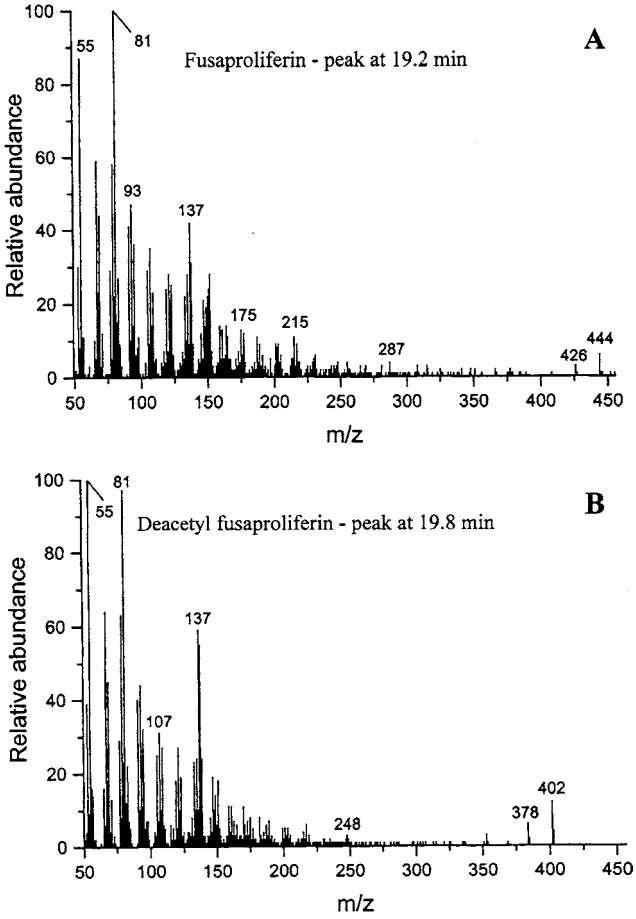

Figure 5. Electron impact mass spectra of the fusaproliferin derivatives separated on the RTX-65 GC column shown in **Figure 4**. (A) Mass spectrum of the fusaproliferin peak eluting at 19.2 min (**Figure 4A**). (B) Mass spectrum of deacetyl-fusaproliferin peak eluting at 19.8 min (**Figure 4B**).

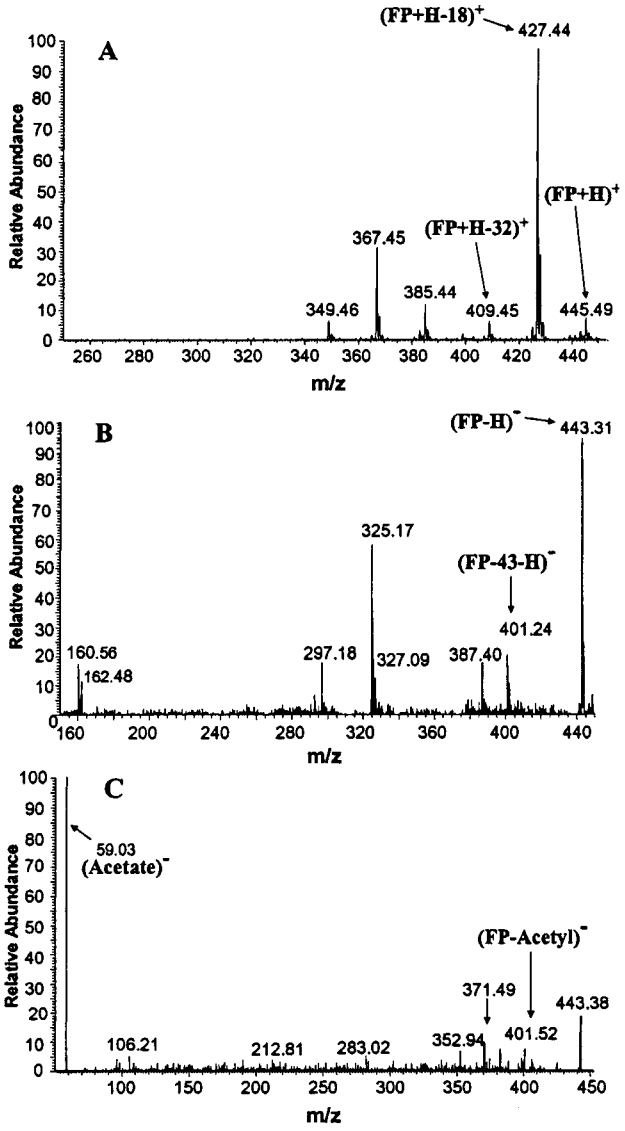

Figure 6. Electrospray ionization mass spectrometry of the purified fusaproliferin. (A) Positive-ion ESI full-scan mass spectrum. (B) Negative-ion ESI full-scan mass spectrum. (C) Negative ESI product-ion spectra of the negative charged parent ion at *m/z* 443.3 from **Figure 6B**.

at *m/z* 443.3 $(M - H)^-$ and *m/z* 401.2 (**Figure 6B**). The fragment at *m/z* 401.2 probably corresponds to loss of an acetyl and a hydrogen $(M - 43 - H)^-$. Pocsfalvi et al. (*19*) have reported a similar negative-ion mass spectrum, although they did not show the ions below *m/z* 300. It was their conclusion that the presence of the negative ion at *m/z* 401 indicated that the fusaproliferin was contaminated with deacetyl-fusaproliferin.

To further investigate that possibility, we evaluated negative-ion electrospray product ions from the negative parent ion shown in **Figure 6B** (*m/z* 443.3). Results of the MS/MS ESI data shown in **Figure 6C** indicate that the deacetyl-fusaproliferin is produced in the ion source as noted by the ion at *m/z* 401.5. Furthermore, we saw a large apparent acetate peak at *m/z* 59.0 that would have been produced by the deacetylation of the fusaproliferin. Also, if there had been deacetyl-fusaproliferin in our sample, it would have been readily apparent by HPLC and GC/MS, which was not the case.

The ^{1}H NMR spectrum of fusaproliferin was essentially the same as those reported by Randazzo et al. (*3*), Ritieni et al. (*4*), and Manetti et al. (*20*), which provides further evidence of the purity of our material.

Stability of Fusaproliferin During Storage. The storage stability of fusaproliferin is crucial for both further research with fusaproliferin and the development of a fusaproliferin standard. Ritieni et al. (*21*) reported that fusaproliferin could be totally destroyed at a temperature of 240 °C. We found that fusaproliferin readily deacetylates to form deacetyl-fusaproliferin at room temperature (25 °C) and above. When we first checked the purified fusaproliferin by HPLC with the analytical C18 column, there was no detectable deacetyl-fusaproliferin. After concentration on the evaporator at 45 °C for 20 min, a small peak of deacetyl-fusaproliferin began to appear in the HPLC chromatogram (**Figure 3**, inset). Thus, a small proportion of the fusaproliferin decomposes to deacetyl-fusaproliferin just during the concentration step. The peak area of the fusaproliferin component dropped to 92.0 ± 0.63% (mean ± SD) of the total peak areas after frequent exposure to room temperatures (~20 °C) for 1 month. The peak area of the fusaproliferin component remained at 92.0 ± 0.07% of the total peak areas at the end of another 2 months and at 91.7 ± 0.07% at the end of the fourth month. However, the decomposition process was negligible over a 1-year period when the fusaproliferin standard was kept frozen (−20 °C). Thus, freezing can be used to maintain fusaproliferin standard stability for at least several months.

ABBREVIATIONS USED

FP, fusaproliferin; deacetyl-FP, deacetyl-fusaproliferin.

ACKNOWLEDGMENT

We express special thanks to Antonio Logrieco for providing the fusaproliferin reference material. We thank Amy Beyer and Brooke van Scoyc for technical assistance. This is Contribution No. 02-180-J from the Kansas Agricultural Experiment Station, Manhattan, KS.

LITERATURE CITED

(1) FDA Website. Guidance for industry: Fumonisin levels in human foods and animal feeds. Final Guidance, Nov 2001. http://www.fda.gov/OHRMS/DOCKETS/98fr/001277gd.pdf (accessed Dec 2002).

(2) D'Mello, J. P. F.; Placinta, C. M.; Macdonald, A. M. C. *Fusarium* mycotoxins: A review of global implications for animal health, welfare and productivity. *Anim. Feed Sci. Technol.* **1999**, *80*, 183−205.

(3) Randazzo, G.; Fogliano, V.; Ritieni, A.; Mannina, L.; Rossi, E.; Scarallo, A.; Segre, A. L. Proliferin, a new sesterterpene from *Fusarium proliferatum*. *Tetrahedron* **1993**, *49*, 10883−10896.

(4) Ritieni, A.; Fogliano, V.; Randazzo, G.; Scarallo, A.; Logrieco, A.; Moretti, A.; Mannina, L.; Bottalico, A. Isolation and characterization of fusaproliferin, a new toxic metabolite from *Fusarium proliferatum*. *Nat. Toxins* **1995**, *3*, 17−20.

(5) Santini, A.; Ritieni, A.; Fogliano, V.; Randazzo, G.; Mannina, L.; Logrieco, A.; Benedetti, E. Structure and absolute stereochemistry of fusaproliferin, a toxic metabolite from *Fusarium proliferatum*. *J. Nat. Prod.* **1996**, *59*, 109−112.

(6) Logrieco, A.; Moretti, A.; Fornelli, F.; Fogliano, V.; Ritieni, A.; Caiaffa, M. F.; Randazzo, G.; Bottalico, A.; Macchia, L. Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina*, SF-9 insect cells, and IARC/LCL 171 human B-lymphocytes. *Appl. Environ. Microbiol.* **1996**, *62*, 3378−3384.

(7) Ritieni, A.; Monti, S. M.; Randazzo, G.; Logrieco, A.; Moretti, A.; Peluso, G.; Ferracane, R.; Fogliano, V. Teratogenic effects of fusaproliferin on chicken embryos. *J. Agric. Food Chem.* **1997**, *45*, 3039−3043.

(8) Moretti, A.; Logrieco, A.; Ritieni, A.; Randazzo, G.; Macchia, L. Beauvericin and fusaproliferin: Emerging *Fusarium* toxins. *Bull. Inst. Compr. Agric. Sci. Kinki Univ.* **1998**, *6*, 13-21.

(9) Fotso, J.; Leslie, J. F.; Smith, J. S. Production of beauvericin, moniliformin, fusaproliferin, and fumonisins B_1, B_2, and B_3 by fifteen ex-type strains of *Fusarium* species. *Appl. Environ. Microbiol.* **2002**, *68*, 5195−5197.

(10) Srobarova, A.; Moretti, A.; Ferracane, R.; Ritieni, A.; Logrieco, A.; Toxigenic *Fusarium* species of *Liseola* section in pre-harvest maize ear rot, and associated mycotoxins in Slovakia. *Eur. J. Plant Pathol.* **2002**, *108*, 299−306.

(11) Munkvold, G.; Stahr, H. M.; Logrieco, A.; Moretti, A.; Ritieni, A. Occurrence of fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. *Appl. Environ. Microbiol.* **1998,** *64*, 3923−3926.

(12) Shephard, G. S.; Sewram, V.; Nieuwoudt, T. W.; Marasas, W. F. O.; Ritieni, A. Production of the mycotoxins fusaproliferin and beauvericin by South African isolates in the *Fusarium* section Liseola. *J. Agric. Food Chem.* **1999**, *47*, 5111−5115.

(13) Castellá, G.; Munkvold, G. P.; Imerman, P.; Hyde, W. G. Effects of temperature, incubation period and substrate on production of fusaproliferin by *Fusarium subglutinans* ITEM 2404. *Nat. Toxins* **1999**, *7*, 129−132.

(14) Kostecki, M.; Wisniewska, H.; Perrone, G.; Ritieni, A.; Golinski, P.; Chelkowski, J.; Logrieco, A. The effects of cereal substrate and temperature on production of beauvericin, moniliformin and fusaproliferin by *Fusarium subglutinans* ITEM-1434. *Food Addit. Contam.* **1999**, *16*, 361−365.

(15) Ritieni, A.; Moretti, A.; Logrieco, A.; Bottalico, A.; Randazzo, G.; Monti, S. M.; Ferracane, R.; Fogliano, V. Occurrence of fusaproliferin, fumonisin B_1, and beauvericin in maize from Italy. *J. Agric. Food Chem.* **1997**, *45*, 4011−4016.

(16) Correll, J. C.; Klittich, C. J. R.; Leslie, J. F. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* **1987**, *77*, 1640−1646.

(17) Monti, S. M.; Fogliano, V.; Logrieco, A.; Ferracane, R.; Ritieni, A. Simultaneous determination of beauvericin, enniatins, and fusaproliferin by high performance liquid chromatography. *J. Agric. Food Chem.* **2000**, *48*, 3317−3320.

(18) Sewram, V.; Nieuwoudt, T. W.; Marasas, W. F. O.; Shephard, G. S.; Ritieni, A. Determination of the *Fusarium* mycotoxins, fusaproliferin and beauvericin by high-performance liquid chromatography−electrospray ionization mass spectrometry. *J. Chromatogr. A* **1999**, *858*, 175−185.

(19) Pocsfalvi, G.; Ritieni, A.; Randazzo, G.; Dobo, A.; Malorni, A. Interaction of *Fusarium* mycotoxins, fusaprolierin and fumonisin B_1, with DNA studied by electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 5795−5801.

(20) Manetti, C.; Fogliano, V.; Ritieni, A.; Santini, A.; Randazzo, G.; Logrieco, A.; Mannina, L.; Segre, A. L. Determination of the structure of fusaproliferin by ^{1}H NMR and distance geometry. *Struct. Chem.* **1995**, *6*, 183−189.

(21) Ritieni, A.; Monti, S. M.; Moretti, A.; Logrieco, A.; Gallo, M.; Ferracane, R.; Fogliano, V. Stability of fusaproliferin, a mycotoxin from *Fusarium* spp. *J. Sci. Food Agric.* **1999**, *79*, 1676−1680.

Received for review August 21, 2002. Revised manuscript received October 31, 2002. Accepted November 1, 2002. This research was supported in part by the Sorghum and Millet Collaborative Research Support Program (INTSORMIL) AID/DAN-1254-G-00-0021-00 from the U.S. Agency for International Development (USAID) and the Cooperative State Research, Education, and Extension Service, United States Department of Agriculture, under Agreement No. 93-34211-8362.

JF020904Z