Salmonella typhimurium TA100, TA102, and TA104". *Environ. Mutag.* **1987**, in press.
Soltys, P. A.; Mauney, T.; Natusch, D. F. S.; Schure, M. R. "Time-Resolved Solvent Extraction of Coal Fly Ash: Retention of Benzo[a]pyrene by Carbonaceous Components and Solvent Effects". *Environ. Sci. Technol.* **1986**, *20*, 175–180.
Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics*; McGraw-Hill: New York, 1960.
Tappel, A. L. "Protection against Free Radical Liquid Peroxidation Reactions". *Adv. Exp. Med. Biol.* **1978**, *97*, 111–131.
Yahagi, T.; Nagao, M.; Seino, Y.; Matsushima, T.; Sugimura, T.; Okada, M. "Mutagenicities of N-Nitrosamines on Salmonella". *Mut. Res.* **1977**, *48*, 121–130.

Received for review February 9, 1987. Revised manuscript received July 7, 1987. Accepted October 14, 1987.

Fluorescence Detection of Phototoxic Psoralens in Vegetable Products

Tuan Vo-Dinh,* D. Allen White, Michael A. O'Malley, Paul J. Seligman, and Ross C. Beier

This work describes the fluorescence detection of phototoxic psoralens in various vegetable products including celery, lime, carrot, parsley, and spinach. The spectral properties of psoralen, 5-methoxypsoralen, and 8-methoxypsoralen and raw extracts of celery and carrots are discussed in detail. The measurements used a portable fiberoptics luminoscope developed for monitoring surface and skin contamination.

I. INTRODUCTION

Recently there has been increasing interest in psoralens (linear furanocoumarins) because of their photobiological properties. DNA lesions caused by psoralen or its derivatives in conjunction with long-wavelength ultraviolet (UV) radiation have been investigated (Hearst, 1981). Psoralens were found to intercalate at two adjacent base pairs of DNA and form covalent bonds to a pyrimidine base on each DNA strand upon UV radiation, thus generating a cross-link. Psoralens, especially 8-methoxypsoralen (8-MOP), have been used in the treatment of various skin diseases such as psoriasis (Parrish et al.,-1974) and leukoderma (Scott et al., 1976). Psoralens occur naturally in a number of plants from several families and account for the phototoxicity associated with various species such as celery and plants such as parsnip roots (Beier and Oertli, 1983; Oertli et al., 1984). Chromatographic techniques have been developed to analyze linear furanocoumarins in celery (Beier et al., 1983; Beier, 1985). A recent study has investigated dermatitis in grocery workers associated with high concentrations of furanocoumarine in celery (Berkley et al., 1986; Seligman et al., 1987). Detection of psoralens in human foods is a critical step in assessing the importance of human exposure to phototoxic psoralens. This paper reports results from fluorescence measurements designed to detect psoralens directly in food plant liquid extracts. Emphasis is on surface and skin contamination of workers who are frequently involved in routine handling of vegetables or food plants. A fiberoptics luminoscope developed for monitoring skin contamination of workers in various industrial settings (Vo-Dinh and Gammage, 1981; Vo-Dinh, 1987) was used to measure the surface fluorescence of various products from food plants. Spectral characteristics of psoralen, 5-methoxypsoralen (5-MOP), and 8-MOP and raw extracts of celery and carrots have been investigated and will be discussed in detail. The results suggest that the use of direct fluorescence detection with the luminoscope has great potential for assessing the problems associated with skin contamination by phototoxic psoralens contained in food plants and vegetables.

Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831 (T.V.-D., D.A.W.), National Institute for Occupational Safety and Health, Cincinnati, Ohio 45226 (M.A.M., P.J.S.), and Veterinary Toxicology and Entomology Research Laboratory, U.S. Department of Agriculture—Agricultural Research Service, College Station, Texas 77841 (R.C.B.).

II. EXPERIMENTAL SECTION

2.1. Fiberoptics Luminoscope. A hand-held fiberoptics-based luminoscope was developed to conduct investigation of skin contamination among workers in energy technologies (Vo-Dinh, 1987). This instrument was further developed with extended capabilities for quantitatively measuring luminescent contaminants on skin or on solid surfaces. The detailed design and electronic circuitry of an improved version are given elsewhere (Vo-Dinh, 1987; Vo-Dinh and White, 1986). A field-portable version of this instrument is commercially available from Environmental Systems Corp., Knoxville, TN 37912. Only the basic features of the instrument are given here. The excitation source was a 125-W mercury lamp commercially available (PBL Electro-Optics, Inc., Model Hg-125). The filter holder, which accommodated an easily interchangeable interference filter with transmission in the UV, was attached to the entrance aperture of a bifurcated fiberoptics waveguide (Ealing Corp.) that was used to channel the exiting light onto the surface being scanned and to retransmit the luminescence of the contaminants back onto the entrance slit of the detection system. In this new instrument a holographic monochromator was used to select the spectral region for detection. Luminescence signals were detected by a photomultiplier (Research Support Instruments, Inc.) that operated in the digital single-photon counting mode. The signal recording, background-nulling, and data conversion system was designed and fabricated at Oak Ridge National Laboratory (ORNL).

2.2. Reagents and Materials. Linear furanocoumarins used in this study were obtained from the following sources: psoralen, Interchem. Corp., Paramus, NJ; 5-MOP, Aldrich Chemical Co., Inc., Milwaukee, WI; 8-MOP, Biochemical Laboratories, Redondo Beach, CA. Crystalline psoralen, 5-methoxypsoralen, and 8-methoxypsoralen were provided by NIOSH. Spectroscopic grade ethanol was used to prepare solutions of these compounds. Fluores-

0021-8561/88/1436-0333$01.50/0 © 1988 American Chemical Society

cence of solutions was measured by a Perkin-Elmer Model 650-40 spectrophotometer using quartz cuvettes. Spectral fluorescence measurements employing a paper substrate were performed on a Perkin-Elmer Model MPF-43A spectrophotometer. The paper substrate used was Whatman No. 42 filter paper positioned in finger-type cell holders. Vegetables used in this study were obtained from a local grocer.

2.3. Procedure. Luminoscope measurements were made of vegetable materials suspected of contributing to the problem of photosensitized psoralen rashes. This simply involved scratching or rubbing of the various materials on the surface of a filter paper disk and subsequent fluorescence measurement of the spot. No sample extraction procedure was required. To perform a measurement, the stethoscopic head of the luminoscope was placed on the spot and the shutter opened to give a reading in photon counts. Measurement of a blank area near each sample spot was taken as the background value. For these experiments the emission monochromator of the luminoscope was set at 428 nm for psoralen and 475 nm for 5-MOP and 8-MOP. A broad-band filter transmitting light at 360 nm with a 50-nm bandwidth selected the excitation wavelengths from the mercury source.

Excitation and emission spectra were obtained on a Perkin-Elmer spectrometer 650-40 for ethanolic solutions of psoralen, 5-MOP, and 8-MOP. The concentration of the psoralen solution was 10 μg/mL; concentrations of the 5-MOP and 8-MOP solutions were both 1 μg/mL.

With the excitation maxima determined from the fluorescence measurement of the above solution, a series of fluorescence emission measurements was performed with use of the solutions applied to a filter paper substrate. In this case the concentration of all solutions of the psoralen compounds was 100 μg/mL. A 2.5-μL aliquot of each solution was spotted and allowed to dry on small filter paper disks held by finger-type sample cells designed to fit into the sample compartment of the spectrometer for spectroscopic analysis. The excitation wavelength used to analyze psoralen was 342 nm; 317-nm excitation was used for 5-MOP and 8-MOP.

For comparative purposes similar fluorescence measurements were made of juices from carrot and celery. Juices from peelings and scrapings were absorbed into the filter paper disks by direct contact and allowed to dry. Additionally, celery leaves were shredded and soaked in ethanol to provide a filtered solvent extract. A 2.5-μL spot of this extract served as a third vegetable sample. Analysis of these samples was performed with an excitation wavelengths at 342 and 317 nm.

III. RESULTS AND DISCUSSION

3.1. Fluorescence of Psoralens and Derivatives. Measurements were performed on the Perkin-Elmer spectrometer to measure the fluorescence characteristics of psoralen, 5-MOP, and 8-MOP. Figure 1 shows the fluorescence emission and excitation spectra of psoralen. The sample consisted of a 10 mg/mL ethanolic solution of psoralen. The spectra of Figure 1 show that psoralen exhibits a broad-band and featureless emission having a maximum intensity at about 429 nm. The fluorescence excitation spectrum of psoralen exhibits three peaks at 258, 308, and 340 nm. The spectral properties of substituted psoralens have been previously studied (Mantulin and Song, 1973). The fluorescent states of psoralen have been assigned to a $\pi\pi^*$ configuration on the basis of molecular orbital calculations and their spectral structures. It has been suggested that the formation of the monoadduct between the thymine base of DNA and psoralens occurs via the first excited singlet state (Beaumont et al., 1980).

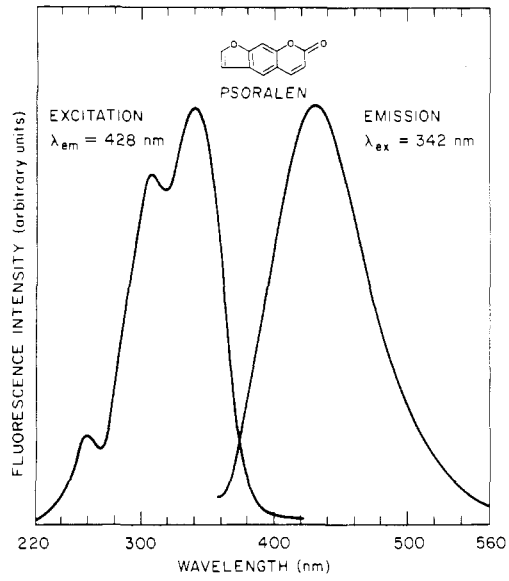

Figure 1. Fluorescence emission and excitation spectra of psoralen in ethanol (10 μg/mL).

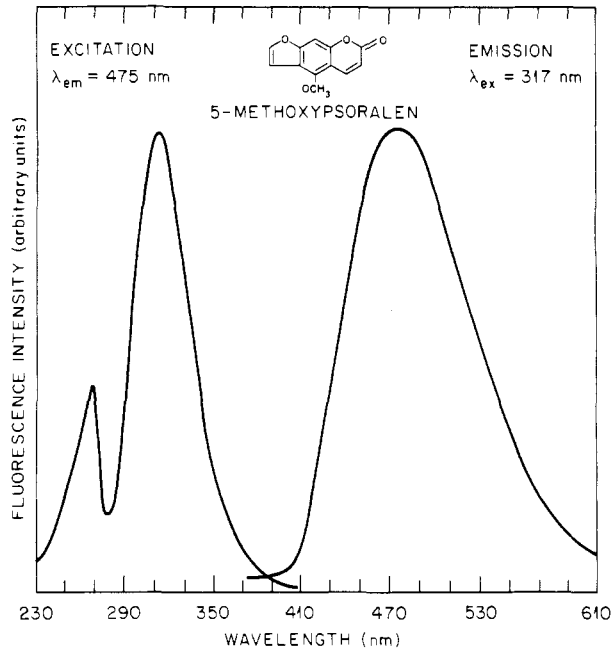

Figure 2. Fluorescence emission and excitation spectra of 5-methoxypsoralen in ethanol (10 μg/mL).

Figures 2 and 3 show the fluorescence emission and excitation spectra of 5-MOP (1 μg/mL in ethanol) and 8-MOP (1 μg/mL in ethanol). The two methoxy derivatives of psoralen exhibit similar spectral characteristics, with the maximum emission bands at about 475 nm for 5-MOP and 8-MOP. Compared to the parent compound, psoralen, the fluorescence emission spectra of 5-MOP and 8-MOP are red-shifted by about 46 nm. The excitation spectra of Figures 2 and 3 indicate that the excitation maxima of 5-MOP and 8-MOP are blue-shifted by 22 nm with respect to psoralen. These results show that it is possible to differentiate psoralen from 5-MOP and 8-MOP by using different excitation and emission wavelengths.

It is of interest to note that the fluorescence intensity of 8-MOP depends on the polarity of the solvent. These observations are in agreement with the results reported previously on the increased fluorescence of 8-MOP in polar solvents (Beaumont et al., 1980). This phenomenon is

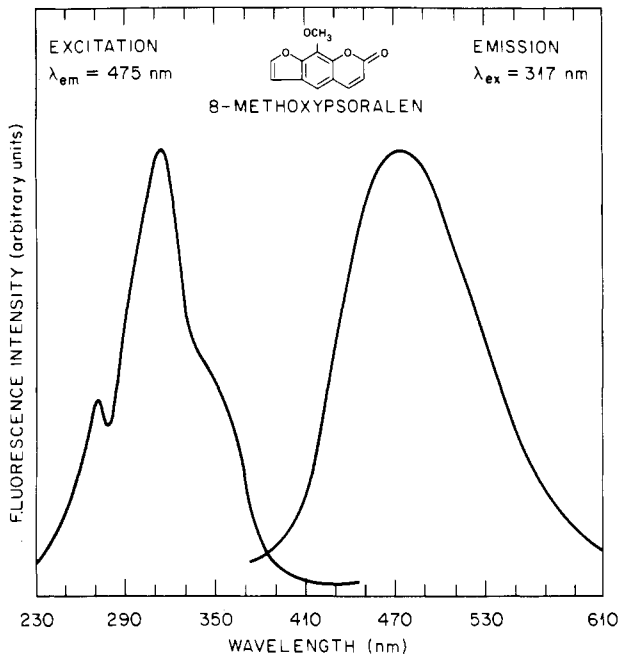

Figure 3. Fluorescence emission and excitation spectra of 8-methoxypsoralen in ethanol (10 μg/mL).

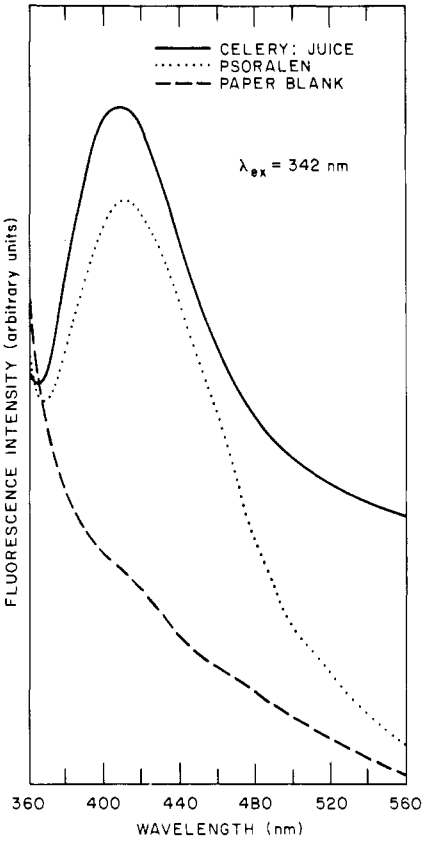

Figure 4. Fluorescence emission spectra of celery raw liquid extract and psoralen adsorbed on filter paper.

often referred to as the "solvent effect". When the dipole moment of the solute molecule decreases upon an electronic transition, the corresponding band is shifted to a shorter wavelength (blue-shift) with increasing solvent polarity. This occurs for $n\pi^*$ transitions. The increase in fluorescence yield in polar solvents can be explained by a blue-shift of the triplet $n\pi^*$ state above the singlet (fluorescent) $n\pi^*$ state. This shift would decrease the radiationless transition from the fluorescent singlet state to the phosphorescent triplet state, thus increasing the

Table I. Fluorescence Intensities of Psoralen, 5-MOP, and 8-MOP Detected by the Luminoscope

		net fluorescence signal, arbitrary units	
compound	amt, ng	Whatman 42 paper	acetylated paper
psoralen[a]	250	550	w[c]
	25	50	
5-MOP[b]	250	300	20
	25	30	
8-MOP[b]	250	30	w

[a] λ_{em} = 428 nm; λ_{ex} = 365 nm. [b] λ_{em} = 475 nm; λ_{ex} = 365 nm. [c] w = weak signal, not quantifiable.

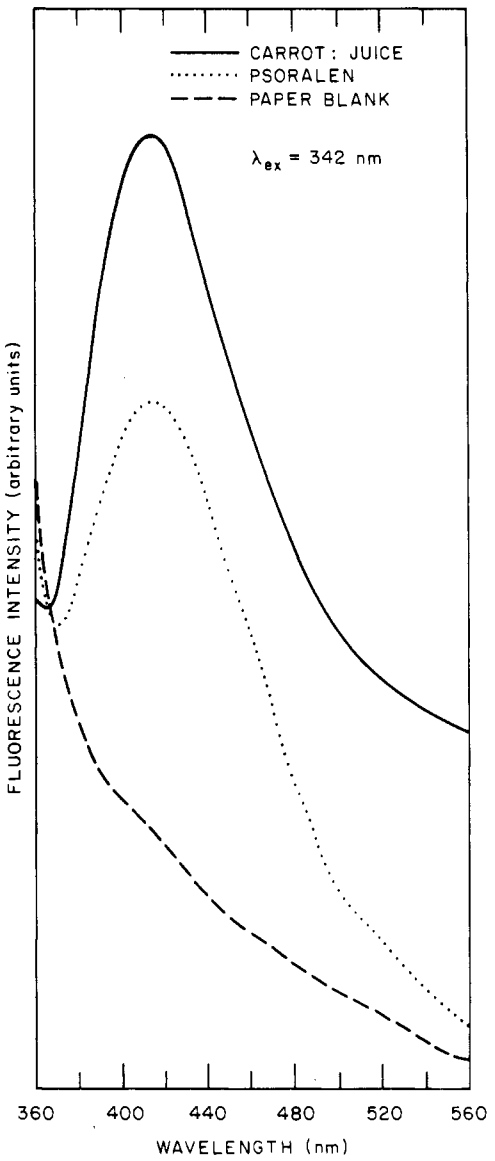

Figure 5. Fluorescence emission spectra of carrot raw liquid extract and psoralen adsorbed on filter paper.

fluorescence emission probability. This effect was confirmed by decreased fluorescence signal of psoralen, 5-MOP, and 8-MOP adsorbed on acetylated paper that contains nonpolar groups (Table I). Nontreated Whatman No. 42 paper was therefore used in subsequent measurements.

3.2. Fluorescence of Vegetable Products. Fluorescence spectra of fresh celery and carrots were measured to detect the presence of psoralen and its methoxy derivatives. Figures 4 and 5 show the fluorescence spectra of juices of celery and carrot adsorbed on filter paper with

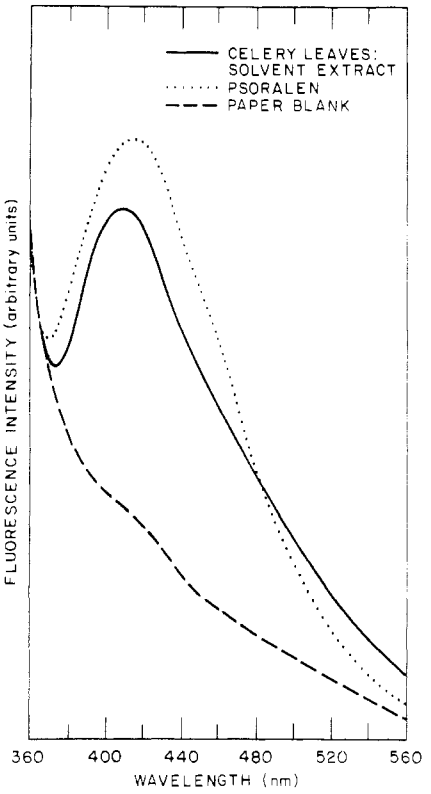

Figure 6. Fluorescence emission spectra of celery leaf ethanol extract and psoralen adsorbed on filter paper.

342 nm as the excitation wavelength. For comparison, the fluorescence spectrum of psoralen absorbed on filter paper is also given in these two spectra. The results indicate that the fluorescence of the juices exhibits an emission similar to that of psoralen. Note that the fluorescence of psoralen adsorbed on filter paper is similar to that of psoralen in ethanol solution (Figure 1). Figure 6 shows the fluorescence spectrum of an ethanol extract from celery spotted on filter paper. The results indicate that the ethanol extract also exhibits a fluorescence emission similar to that of psoralen.

Measurements were conducted with the 315-nm excitation wavelength in order to detect the presence of 5-MOP and 8-MOP. The results are shown in Figure 7 for celery and carrot juice. Although the 315 nm was optimized to 5-MOP and 8-MOP, the fluorescence spectra of the vegetable products did not reveal the presence of these two psoralen derivatives. This could be due to the fact that the fluorescence spectra of 5-MOP and 8-MOP are masked by other fluorescing components emitting at shorter wavelength.

3.3. Surface Fluorescence Detection Using the Fiberoptics Luminoscope. After detailed investigations of the fluorescence characteristics of celery and carrot products in order to confirm the presence of psoralen in these vegetables, measurements were performed with the fiberoptics luminoscope to illustrate the potential of this device for measurement of surface contamination by these products. The results illustrated in Table II indicated that the luminoscope can be used to detect a variety of fresh vegetable liquid extracts adsorbed into filter paper pads. It is of interest to note that the fluorescence of these vegetable liquid extracts can also be detected directly on the skin although the emission intensity is decreased. This could be attributed to the fact that the skin surface is less "polar" than the untreated Whatman No. 42 filter paper used for fluorescence measurement. The possibility for direct detection of skin contamination of psoralens from

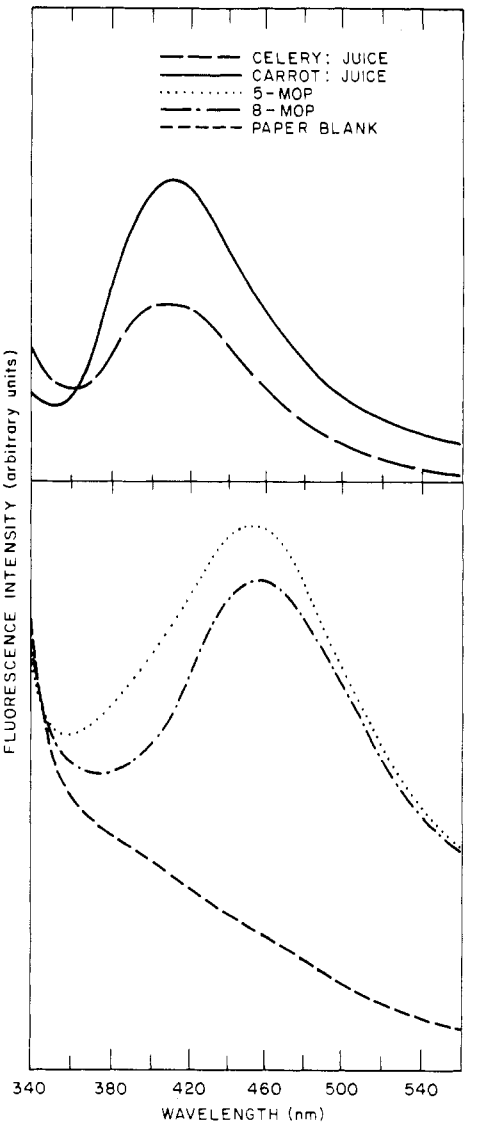

Figure 7. Fluorescence emission spectra (λ_{ex} = 317 nm) of celery and carrot raw liquid extracts, 5-methoxypsoralen, and 8-methoxypsoralen (λ_{ex} = 317 nm).

Table II. Detection of Fluorescence from Vegetable Juices on Filter Paper by the Luminoscope

juice	net signal[a]	juice	net signal[a]
celery base	4050	carrot leaf	w[b]
celery leaf	990	carrot root	2670
celery blade	750	parsley	w
celery skin	630	spinach	w
lime skin	350		
lime juice	2350		

[a] Fluorescence intensity in arbitrary units; λ_{ex} = 365 nm; λ_{em} = 480 nm. [b] w = weak signal, not quantifiable.

vegetable products is under investigation.

IV. CONCLUSION

This study shows that fluorescence can be used to detect the presence of psoralens in a variety of vegetable products. Although the fluorescence spectra are broad and featureless and the vegetables may contain many other compounds that fluoresce, this technique can be used as a screening tool to detect potential contamination by these phototoxic agents. Of special interest is the development of special filter pads worn by workers for detecting occupational skin contamination. Another area of great research interest and potential application is the direct detection of these materials on skin using a portable device

such as the fiberoptics luminoscope skin monitor.

Registry No. 5-MOP, 484-20-8; 8-MOP, 298-81-7; psoralen, 66-97-7.

LITERATURE CITED

Beaumont, P. C.; Parsons, B. J.; Navaratnan, S.; Phillips, G. O. Allen, J. C. *Biochim. Biophys. Acta* **1980**, *608*, 259.
Beier, R. C. *J. Liq. Chromatogr.* **1985**, *8*, 1923.
Beier, R. C.; Oertli, E. H. *Phytochemistry* **1983**, *22*, 2595.
Beier, R. C.; Ivie, W. G.; Oertli, E. H.; Holt, D. L. *Food Chem. Toxicol.* **1983**, *21*, 163.
Berkley, S. F.; Hightower, A. W.; Beier, R. C.; Fleming, D. W.; Brokopp, C. D.; Ivie, G. W.; Broome, C. V. *Ann. Int. Med.* **1986**, *105*, 351.
Hearst, J. E. *Ann. Rev. Biophys. Bioeng.* **1981**, *10*, 69.
Mantulin, W. W.; Song, P. S. *J. Am. Chem. Soc.* **1973**, *95*, 5122.
Oertli, E. H.; Beier, R. C.; Ivie, G. W.; Rowe, L. D. *Phytochemistry* **1984**, *23*, 439.
Parrish, J. A.; Fitzpatrick, T. B.; Tannenbaum, L.; Pathak, M. A. *New Engl. J. Med.* **1974**, 1207.
Scott, B. R.; Pathak, M. A.; Mohn, G. R. *Mutat. Res.* **1976**, 29.
Seligman, P. J.; Mathias, C. G. T.; O'Malley, M. A.; Beier, R. C.; Fehrs, L. J. *Arch. Dermatol.* **1987**, *123*, 1478.
Vo-Dinh, *Am. Ind. Hyg. Assoc. J.* **1987**, *48*, 594.
Vo-Dinh, T.; Gammage, R. B. *Am. Ind. Hyg. Assoc. J.* **1981**, *42*, 112.
Vo-Dinh, T.; White, D. A. *Anal. Chem.* **1986**, *58*, 1128.

Received for review September 12, 1986. Accepted September 17, 1987. Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

Analysis of Ergopeptine Alkaloids in Endophyte-Infected Tall Fescue

Shelly G. Yates* and Richard G. Powell

A method has been developed for analysis of ergopeptine alkaloids in tall fescue by means of high-performance liquid chromatography (HPLC) with fluorescence detection. Ergopeptine alkaloids in methanol extracts of seed were routinely analyzed in an acetonitrile–pH 7.6 buffer gradient with dihydroergocristine as an internal standard. Measured recovery of ergovaline added at the 1.5 μg/g level was 98%, and the lower limit of detection was 150 pg. Ergopeptine alkaloids, primarily ergovaline, are presumed contributors to animal toxicity of tall fescue (*Festuca arundinacea*) infected with *Acremonium coenophialum*. The method complements tandem mass spectrometry (MS–MS) in that HPLC readily distinguishes physiologically active "ine" and inactive "inine" epimers.

Tall fescue (*Festuca arundinacea* Schreb.) was brought to the new world by early settlers and existed in relative obscurity for many years. A pure stand of a naturalized variety was observed in Kentucky in 1931, and this variety, KY-31, was released commercially in 1943 (Ball, 1984) and recommended for use by the Department of Agriculture. Tall fescue gained rapidly in popularity (Cowan, 1956), and recently forage scientists estimated that 35 million acres are grown in the southeastern United States (Ball, 1984). Much of the time, tall fescue compares favorably with other pasture grasses with respect to daily weight gain of cattle on pasture. Under heat stress during summer months, however, clinical signs of fescue toxicity, including elevated temperature, poor weight gain, problems with reproduction, and reduced lactation (Bush et al., 1979), are often observed. This phenomenon, referred to as summer slump or summer syndrome, has become accepted as normal by livestock producers who rely primarily on fescue forage. During periods of stress due to cold in winter, another syndrome known as fescue foot can become important (Bush et al., 1979). Characteristic signs of fescue foot are loss of weight, rough hair coat, arched back, trembling, and gangrene of the extremities in severe cases. Despite these reports and estimated economic losses of $50 to $200 million annually (Siegel et al., 1984), tall fescue is favored as a cool season pasture for year-around grazing systems. The problems with fescue can be largely avoided by proper management practices. Full understanding of the causes of these syndromes remains elusive due to lack of understanding of all contributing factors and the need for rapid and sensitive analytical procedures.

Symptoms of fescue toxicity often mimic those of ergotism; however, ergot (*Claviceps purpurea*) does not appear to be the major problem in fescue pastures. In recent years it has been recognized that 95% or more of the tall fescue grown in the United States is infected, to varying degrees, with *Acremonium coenophialum* and that the degree of toxicity of fescue forage is related to the degree of infection by this endophytic fungus. *A. coenophialum* has been demonstrated to produce ergopeptine alkaloids, notably ergovaline (Porter et al., 1981; Yates et al., 1985), and, though not present in endophyte-free fescue, ergovaline appears to be a normal constituent of endophyte-infected fescue leaf blades and sheaths at levels up to 2.8 ppm (Lyons et al., 1986).

The presence of ergovaline and other ergot alkaloids in fescue at reported levels is of concern due to their wide range of potent physiological activities (Berde and Schild, 1978). In addition, concentrations of these alkaloids in forage may fluctuate widely due to seasonal or environmental changes (Lyons et al., 1986), unrecognized analogues may be present, and additive or synergistic effects involving other types of compounds may be complicating factors. This report describes a rapid, sensitive, and reasonably quantitative method for analysis of ergot alkaloids in tall fescue. The procedure is a modification of an HPLC method for analysis of ergot alkaloids in flour (Scott and

Northern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, Peoria, Illinois 61604.

This article not subject to U.S. Copyright. Published 1988 by the American Chemical Society