Steroid Metabolites of *Acremonium coenophialum*, an Endophyte of Tall Fescue

Norman D. Davis,* Richard J. Cole, Joe W. Dorner, John D. Weete, Paul A. Backman, Edward M. Clark, Charles C. King, Stephen P. Schmidt, and Urban L. Diener

Three *Acremonium coenophialum* metabolites, ergosterol, ergosterol peroxide, and ergosta-4,6,8-(14),22-tetraen-3-one, were isolated from field-grown, fungus-infected (toxic) tall fescue and mycelium grown in vitro. Mycelium contained 10 μg of total sterol/mg dry weight of tissue, with ergosterol comprising 40% of the total sterols. Mycelium contained EP only when grown in the light. EP was toxic to brine shrimp, two bacterial species, and chick embryos. Whether EP is involved in tall fescue toxicity is not known, but it was the only toxic compound found in chloroform–methanol extracts from both fungus-infected fescue and laboratory-grown mycelium and EP was not found in nontoxic tall fescue.

Cattle grazed on Kentucky 31 tall fescue (*Festuca arundinaceae* Schreb.) during the summer often exhibit symptoms known collectively as "summer syndrome". These symptoms include reduced weight gain, rough hair coat, slightly elevated temperature, excessive salivation, rapid breathing, and excitability (Hoveland et al., 1983). Although summer syndrome was previously associated with hot weather, recent research has shown that poor weight gain occurs throughout the grazing season when cattle feed on tall fescue infected with an endophytic fungus (Hoveland et al., 1983). Bacon et al. (1977) isolated the endophytic fungus *Epichloe typhina* (Fr.) Tulasne from bent grass. The imperfect (Anamorphic) stage of this fungus is *Acremonium typhinum* Morgan-Jones and Gams. The endophytic fungus isolated from tall fescue in Alabama, Georgia, Kentucky, Texas, and New Zealand (Morgan-Jones and Gams, 1982; Latch et al., 1984; White and Cole, 1985, 1985b) has been classified as *A. coenophialum* Morgan-Jones and Gams; a perfect (teleomorphic) state has never been reported on tall fescue. According to Morgan-Jones (personal communication), the endophytic anamorphs from bent grass and tall fescue differ morphologically as well as in host specificity. In our view, the fungus endophyte of tall fescue is *A. coenophialum* Morgan-Jones and Gams and all other names are erroneous.

It has often been suggested that alkaloids are a likely cause of fescue foot and perhaps other problems associated with tall fescue (Davis and Camp, 1983; Garner et al., 1982; Hemken et al., 1984; Jackson et al., 1981; Jones et al., 19835; Porter et al., 1983). Porter et al. (1979) noted that *E. typhina* (actually *A. coenophialum*) isolated from tall fescue produced ergosine, ergosinine, and chanoclavine I in 28-day-old cultures. Later, Porter et al. (1981), using isobutane chemical ionization mass spectroscopy, reidentified ergosine and ergosinine as ergovaline and ergovalinine. In addition, they identified agroclavine, elymoclavine, penniclavine, and festuclavine from cultures of *E. typhina* (RRC 238), which apparently was *A. coenophialum* and not the *E. typhina* (*A. typhinum*) isolate from bent grass. Recently, Yates et al. (1985), using mass spectrometry/mass spectrometry techniques, detected ergopeptine alkaloids including ergovaline in a sample of tall fescue infected with a fungal endophyte, which they called *E. typhina*, but was also probably *A. coenophialum*. Thus, it is clear that alkaloid metabolites of *A. coenophialum* have been demonstrated to be produced by the fungus in vitro and also in toxic tall fescue. However, no data have been presented to date that demonstrate that these or other ergo peptide alkaloids are the direct or indirect cause of summer syndrome.

The objective of this investigation was to explore alternatives to the ergot alkaloid hypothesis as a cause of summer syndrome, and particularly to examine the spectrum of steroids produced by *A. coenophialum*. We also wanted to determine whether *A. coenophialum* produces any mycotoxins other than alkaloids.

MATERIALS AND METHODS

Culture of the Organism. The *A. coenophialum* strain #ET26 used in this investigation was isolated from toxic tall fescue from Marion Junction, AL, by E. M. Clark and identified according to Morgan-Jones and Gams (1982). The organism was grown in 250-mL Erlemeyer flasks containing 100 mL of nutrient broth consisting of 5% dextrose, 0.5% KH_2PO_4, 0.05% $MgSO_4$, and 0.7% yeast extract (Difco). The fungus, which grew slowly, was incubated as stationary cultures at room temperature (24–27 °C) for up to 90 days.

A. coenophialum was also grown in 500-mL Erlenmeyer flasks containing 100 mL of nutrient broth consisting of 1% dextrose and 0.3% Difco yeast extract for 35 days as stationary cultures at room temperature (24–27 °C). A 10-mL portion of 95% ethanol was added to one set of flasks containing the pregrown mycelium, whereas 10 mL of ethanol plus 10 mg of ergosterol (ER) was added to distilled water in a second set of flasks to serve as controls. All cultures were then incubated under constant shaking at 100 rpm for an additional 30 days at 23 °C. Some cultures of each treatment were kept in the dark, while others were exposed to continuous light from a 40-W incandescent lamp about 26–30 cm from the flasks.

Extract Preparation. Fermented broth was extracted with chloroform, which was evaporated to dryness under an air stream in reduced light. The mycelium was blended in chloroform–methanol (1:1, v/v) in an explosion-proof blender and filtered, and the solvents were evaporated as before. Fresh (or frozen) fescue foliage was blended 3 min in chloroform–methanol (1:1, v/v) and filtered, solvents were evaporated, and the residue was obtained as described above.

Purification of Metabolites. Components of the extracts were separated on 250-μm silica gel TLC plates (Baker 7009-4) by development in chloroform–acetone (95:5, v/v). The air-dried plates were examined under

Department of Botany, Plant Pathology and Microbiology (N.D.D., J.D.W., P.A.B., E.M.C., U.L.D.), Department of Agronomy and Soils (C.C.K.), and Department of Animal and Dairy Sciences (S.P.S.), Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama 36849, and National Peanut Research Laboratory, USDA, ARS, Dawson, Georgia 31742 (R.J.C., J.W.D.).

0021-8561/86/1434-0105$01.50/0

visible and UV light (365 nm) and divided from top to bottom into a series of bands. Each band was scraped from the plate, and the silica gel was washed with methanol and filtered. The solvent was evaporated to dryness under an air stream and the residue obtained as above.

Final purification of ergosterol peroxide (EP) was achieved on a preparative centrifugally accelerated, radial, thin-layer chromatograph (Model 7924 Chromatotron, Harrison Research, Palo Alto, CA). The rotor was coated with a 1-mm layer of silica gel PF and EP was eluted with hexane–ethyl acetate (7:3, v/v) at a flow rate of 4 mL/min. The sample was applied to the rotor in 1 mL of the eluting solvent, and 4-mL fractions were collected and analyzed by TLC. EP eluted in fractions 16–19, which were pooled and concentrated.

Lipid Extraction and Analytical Procedures for Total Sterol Analysis. Lipids were extracted from lyophilized fungus material by the method of Bligh and Dyer (1959). Total sterols were obtained by extracting an alkaline hydrolysate of the total lipid with hexane and analyzed by gas–liquid chromatography as described by Weete et al. (1983), except that a 30-m fused silica capillary column (Applied Science Labs., State College, PA) coated with SE-30 was used at an isothermal oven temperature of 280 °C. Sterols were quantitated by using cholesterol (Sigma, St. Louis, MO) as an external standard, and ER was identified in the samples by comparison of its crude retention time with that of an authentic ER standard (Sigma).

Bioassays. Crude total extracts and components isolated by TLC were tested for toxicity to brine shrimp (*Artemia salina* L.), six species of bacteria, and chick embryos. Except as follows, bioassay procedures were conducted as previously described (Davis et al., 1977). All extracts were taken up in chloroform and sterilized by using a zetapor 0–2 μm disposable filter (AMF CUNO, Meriden, CT). The solvent was removed by evaporation before additions of the brine shrimp–salt solution to each sample well of the test dish. The brine shrimp bioassays were conducted for 24 h with the test dishes covered to reduce evaporation. In the egg bioassays, 10 μL of sterile extract suspended in 80% ethanol was injected via the air sac into 2- to 3-day-old embryonated eggs. The eggs were incubated 18 days and then opened to check for mortality and chick abnormalities. Bacterial bioassays were conducted with paper disks soaked in solutions of EP (0–312 μg/8-mm disk) obtained from crude extracts by preparative TLC procedures. The disks were placed on surfaces of agar plates inoculated with one of six bacterial species are previously described (Wilson, 1985). Plates were incubated 24 h at 30 °C, and zones of inhibition were measured.

RESULTS

Chemistry. *A. coenophialum* mycelium from laboratory cultures contained 10.0 ± 0.2 μg of total sterol/mg dry weight of tissue, about 40% of which was ER as determined by GLC. ER was identified on the basis of its characteristic UV absorption spectrum compared to that of authentic standard (Sigma), i.e. by UV (methanol) [λ_{max} 262, 271, 282, and 293 nm) and also by TLC and GLC as described elsewhere.

Ergosterol peroxide was identified on the basis of comparisons of its UV (end absorption), MS (M^+ at m/e 428), and NMR analyses with those of synthetic EP (Adam et al., 1967; Starratt, 1976). It was not visible on TLC plates under visible or UV light but became gray to black when treated with acid or modified Ehrlich's reagent (Stahl's reagent #74) followed by heat (Stahl, 1969). To confirm the nature of the unknown compound, 500 mg of ergosterol was dissolved in ethyl acetate and allowed to stand at room temperature in the light for 72 h. Formation of breakdown products was observed by TLC, and EP was purified as previously described. UV, MS, and NMR data gathered on the compound thus produced were identical with those of the metabolite purified from fungal cultures, from toxic tall fescue grass, and from the literature (Adam et al., 1967; Gunatilaka et al., 1981; Starratt, 1976).

Table I. Production of Ergosterol, Ergosterol Peroxide (EP), and Ergosta-4,6,8(14),22-tetraen-3-one (ETO) by *Acremonium coenophialum* in Shake Cultures for 30 days

treatment	lt or dk	ergosterol, mg/flask	EP, mg/flask	ETO/flask[a]
control[b]	lt	7.32	<0.03	–
control[b]	dk	11.25	0	–
mycelium[c]	lt	10.00	76.92	+
mycelium[c]	dk	14.29	0	+

[a] Key: –, none detected; +, present but not quantitated. [b] Distilled water, 10 mL of ethanol, 10 mg of ergosterol/flask. [c] A 10-mL portion of ethanol added to 35-day stationary cultures.

Ergosta-4,6,8(14),22-tetraen-3-one (ETO) was identified on the basis of UV absorption λ_{max} (MeOH) 350 nm, TLC, and HPLC properties as reported by Seitz and Paukstelis (1977), by Porter et al. (1975), and by comparisons with an authentic standard obtained from L. M. Seitz. It fluoresced bright green under UV light on TLC plates and turned yellow when sprayed with acid as previously reported by these workers.

Biology. Ergosterol, EP, and ETO were found to be present in variable amounts in samples of toxic tall fescue grass that were known to contain the endophyte but were absent from extracts of tall fescue known to be free of the fungus endophyte. Also, the steroids were found in extracts of *A. coenophialum* mycelium cultured on laboratory medium (Table I). The fermented laboratory medium did not contain any of the three steroid metabolites. Mycelium of *A. ceonophialum* incubated in the dark contained ER and ETO, but not EP. Mycelium incubated in the light contained EP in addition to the other two steroid metabolites. Uninoculated checks spiked with ER and incubated 30 days in the light as well as the dark contained only ER. Thus, EP was not produced from the ER either during incubation or as an artifact in sample extraction and workup.

Brine Shrimp Bioassays. Crude extracts and extract components isolated by TLC from fungus-infected toxic tall fescue, fungus-free nontoxic tall fescue, fermented broth, and mycelium of *A. coenophialum* were bioassayed for toxicity using brine shrimp. None of the samples isolated by TLC were judged to be substantially toxic in a standard 4-h bioassay (data not presented). However, when the bioassay period was extended to 24 h in the dark, toxicity was exhibited by certain samples (Table II). Crude extracts from fungus-infected tall fescue were more toxic than were extracts of noninfected tall fescue. Also, extracts of cultured fungus mycelium, but not the fermented broth, were toxic to the brine shrimp. Symptoms of toxicosis that were observed in this study ranged from stunning to death depending on the amount of concentrated extract present. When stunned, the brine shrimp collected at the bottom of the test wells. They attempted to swim but did not respond to a unilateral incandescent light as did the control organisms. Stunning generally, but not always, was followed by death of the cells. Stunning was a reliable indicator of toxicosis even when mortality did not follow immediately.

When components of the extracts of various samples

Table II. Toxicity of Fungus-Infected Tall Fescue, Noninfected Tall Fescue, *Acremonium coenophialum* Extracts, and Several TLC Bands to Brine Shrimp

sample description	rel tox, 24 h[a]
noninfected tall fescue, crude extract	I
fungus-infected tall fescue, crude extract	III
A. coenophialum mycelium, crude extract	III
A. coenophialum fermented broth	I
toxic tall fescue TLC band	
R_f 0.8–1.0 (ergosta-4,6,8(14),22-tetraen-3-one)	I
R_f 0.6–0.8 (ergosterol and related sterols)	II
R_f 0.4–0.6 (ergosterol peroxide)	III
R_f 0.3–0.4	I
R_f 0.2–0.3	II
R_f 0.0–0.2	I
mycelium TLC band	
R_f 0.9–1.0	I
R_f 0.8–0.9 (ergosta-4,6,8(14),22-tetraen-3-one)	I
R_f 0.6–0.8 (ergosterol and related sterols)	II
R_f 0.4–0.6 (ergosterol peroxide)	III
R_f 0.3–0.4	I
R_f 0.2–0.3	II
R_f 0.0–0.2	I

[a] Percent stunning and/or mortality: 0–20%, I; 21–50%, II; 51–100%, III.

Table III. Toxicity of Tall Fescue and *Acremonium coenophialum* Extracts and Three TLC Bands to Chick Embryos

sample	% mortality[a]
noninfected tall fescue, crude extract	14
fungus-infected tall fescue, crude extract	63
TLC band, R_f 0.25	15
TLC band, R_f 0.45 (ergosterol peroxide)	47
TLC band, R_f 0.65 (ergosterol and related sterols)	16
controls, solvent only	10

[a] Average of three experiments; 28 eggs/sample per experiment.

were separated by preparative TLC and then bioassayed using brine shrimp, it was found that most of the toxicity of the toxic tall fescue could be attributed to EP (Table II). Although the TLC band containing ER was slightly toxic to brine shrimp, this might have been due to some EP formed as an artifact or to a contaminant in the ER band. The ETO isolated on TLC plates was not toxic to brine shrimp (Table II).

Chick Embryo Bioassays. Ergosterol and EP were isolated from TLC plates and further bioassayed for toxicity with chick embryos. The crude extracts of endophyte-infected toxic tall fescue were more toxic to chick embryos than the controls, whereas extracts of noninfected tall fescue were not appreciably toxic (Table III). Also, EP was toxic, whereas ER and the R_f 0.25 material [an unidentified alkaloid (produced a bright blue color after spraying with Ehrlich's reagent)] were only slightly toxic or nontoxic. Thus, as in the case of the brine shrimp bioassays, most of the toxicity observed in crude extracts could be attributed to EP.

Bacterial Bioassays. Growth inhibition of *Serratia marcescens* and *Bacillus megaterium* occurred only at the higher concentrations of EP with 2- and 1-mm zones of inhibition, respectively. Growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Micrococcus luteus* was not inhibited by the extract at any concentration tested.

DISCUSSION

Ergosterol peroxide and ETO were the predominant fungal steroid metabolites in fescue hay and green forage infected with the endophytic fungus *A. coenophialum* and known to be toxic to cattle. ER was the principal steroid component in fungal cultures grown on laboratory medium as it is with most fungi (Weete, 1980). EP was reported by Adam et al. (1967) to be an artifact, and Arditti et al. (1972) supported that contention. However, Starratt (1976) demonstrated that EP can be present in the fungal material and in vitro as a photochemically produced degradation product of ER. Our reseach has shown that *A. coenophialum* produces EP in the light, but not in the dark, and EP is also present in toxic field-grown fescue containing the fungal endophyte. Thus, we support the findings of Starratt (1976) that EP is an authentic photochemically produced fungal component based on our results with *A. coenophialum* and not merely an artifact of the procedures used in isolation. It is not surprising that samples of endophyte-infected fescue contain EP as well as ER and ETO, since the grass was collected from fields exposed to intense sunlight. The fungus produced ER and ETO, in the dark as well as in the light. ETO is produced by numerous fungi (Seitz and Paukstelis, 1977), possibly from Er, and has been found in grasses toxic to cattle (Porter et al., 1975). The total sterol and ER content of *A. coenophialum* is high compared to most fungi (Weete, 1980), and it appears that ER is converted into EP and other products in fungus-infected fescue growing under natural conditions. The relative amounts of these and other sterols may vary depending on the level of infestation, light intensity, and possibly other factors. This relationship is being investigated further.

Ergosterol peroxide was shown to be toxic in three bioassay systems employed in this study. The toxicity of EP, as far as we can determine, has not been previously reported. Although the long-term biological activity of ETO has not been thoroughly investigated, it was found not to be toxic to brine shrimp at the levels tested in this study and is generally regarded as nontoxic (Bacon et al., 1977). At this point, we have not been able to demonstrate a statistical correlation between ER, ETO, and EP levels in infected grass and infection levels of the fungus based on microscopic examination (data to be reported in a separate paper). Since EP and possibly other sterols similar to ergosterol were the only toxic nonalkaloid, chloroform–methanol-soluble fungal metabolites of any consequence common to both toxic tall fescue and laboratory-cultured fungus mycelium, we are continuing our research to determine whether these fungal products are involved in tall fescue toxicity.

ACKNOWLEDGMENT

Thanks are due to D. M. Wilson, L. G. Pounds, and the late Jim Harper of the Mycotoxin laboratory, University of Georgia Experiment Station, Tifton, for aid in obtaining mass spectrometric data and R. H. Cox of the Philip Morris Company, Richmond, VA, for NMR and high-resolution MS data.

Registry No. Ergosterol, 57-87-4; ergosterol peroxide, 2061-64-5; ergosta-4,6,8(14),22-tetraene-3-one, 19254-69-4.

LITERATURE CITED

Adam, H. K.; Campbell, I. M.; McCorkindale, N. J. *Nature* **1967**, *216*, 397.

Arditti, J.; Ernst, R.; Fisch, M. H.; Flick, B. H. *J. Chem. Soc., Chem. Commun.* **1972**, 1217–1218.

Bacon, C. W.; Porter, J. K.; Robbins, J. D.; Luttrell, E. S. *Appl. Environ. Microbiol.* **1977**, *34*, 576–581.

Bligh, E. G.; Dyer, E. G. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.

Davis, C. B.; Camp, B. *J. Vet. Human Toxicol.* **1983**, *25*, 408–411.

Davis, N. D.; Diener, U. L.; Morgan-Jones, G. *Appl. Environ. Microbiol.* **1977**, *34*, 155–157.

Garner, G. B.; Cornell, C. N.; Yates, S. G.; Plattner, R. D.; Rothfus, J. A.; Kwolek, W. K. *J. Anim. Sci.* **1982**, *55*, 185–193.

Gunatilaka, A. A. L.; Gapichand, Y.; Schmidz, F. J.; Djerassi, C. *J. Org. Chem.* **1981**, *46*, 3860–3866.
Hemken, R. W.; Jackson, J. A., Jr.; Boling, J. A. *J. Anim. Sci.* **1984**, *58*, 1011–1016.
Hoveland, C. S.; Schmidt, S. P.; King, Jr., C. C.; Odom, J. W.; Clark, E. M.; McGuire, J. A.; Smith, L. A.; Grimes, H. W.; Holliman, J. L. *Agron. J.* **1983**, *75*, 821–824.
Jackson, J. A., Jr.; Hemken, R. W.; Boling, J. A.; Harmon, R. J.; Buckner, R. C.; Bush, L. P. *J. Dairy Sci.* **1981**, *64*, 152–153.
Jones, T. A.; Buckner, R. C.; Burrus, P. B., II; Bush, L. P. *Crop Sci.* **1983**, *23*, 1135–1140.
Latch, G. C. M.; Christensen, M. J.; Samuels, G. J. *Mycotaxon* **1984**, *20*, 535–550.
Morgan-Jones, G.; Gams, W. *Mycotaxon* **1982**, *15*, 311–118.
Porter, J. K.; Bacon, C. W.; Robbins, J. D. *J. Agric. Food Chem.* **1979**, *27*, 595–598.
Porter, J. K.; Bacon, C. W.; Robbins, J. D.; Betouski, D. *J. Agric. Food Chem.* **1981**, *29*, 653–657.
Porter, J. K. In "Proceedings of Tall Fescue Toxicosis Workshop"; Coop. Ext. Serv., University of Georgia: Athens, GA, 1983; pp 27–33.
Porter, J. K.; Bacon, C. W.; Robbins, J. D.; Higman, H. C. *J. Agric. Food Chem.* **1975**, *23*, 771–775.
Seitz, L. M.; Paukstelis, J. V. *J. Agric. Food Chem.* **1977**, *25*, 833–341.
Stahl, E. "Thin-Layer Chromatography", 2nd ed.; Springer-Verlag: New York, 1969.
Starratt, A. N. *Phytochemistry* **1976**, *15*, 2002–2003.
Weete, J. D. "Lipid Biochemistry of Fungi and Other Organisms"; Plenum Press: New York, 1980.
Weete, J. D.; Sancholle, M. S.; Montant, C. *Biochim. Biophys. Acta* **1983**, *752*, 19–29.
White, J. F., Jr.; Cole, G. T. *Mycologia* **1985a**, *77*, 323–327.
White, J. F., Jr.; Cole, G. T. *Mycologia* **1985b**, *77*, 483–486.
Wilson, M. E. M.S. Thesis, Auburn University, 1984.
Yates, S. G.; Plattner, R. D.; Garner, G. B. *J. Agric. Food Chem.* **1985**, *33*, 719–722.

Received for review January 11, 1985. Revised manuscript received July 15, 1985. Accepted September 25, 1985. AAES Journal No. 6-84728.

High-Performance Liquid Chromatographic Method for Simultaneous Determination of Benomyl and Carbendazim in Aqueous Media

Mikio Chiba* and Raj P. Singh[1]

A simple reversed-phase HPLC method has been developed for the individual determination of benomyl and carbendazim in aqueous media. Benomyl is quantitatively converted to 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole (STB) with NaOH at pH 13 and determined as STB, while carbendazim, present in the sample, is unaffected by this alkaline treatment and determined as carbendazim. Each of two HPLC systems used was run isocratically at a flow rate of 0.8–1.5 mL/min with a UV detector at 280 nm. Two types of 15 cm × 4.6 mm reversed-phase columns (ODS, ODS-II) were used with mobile-phase mixtures consisting of acetonitrile, water, and pH 7 buffer. The minimum detectable limit in solution was 0.03–0.05 μg/mL for STB and carbendazim with a 50-μL injection.

Benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, is a widely used systemic fungicide, but analytical methods for this compound are not yet well developed. Although many papers have been published regarding the analysis of benomyl, the majority of methods published in the past are to determine carbendazim (methyl 2-benzimidazolecarbamate, well-known as MBC), benomyl's most common degradation compound (Figure 1). The major reason for this approach is due to many technical difficulties that are associated with the specific behavior of benomyl in different solvents.

As analytical methods, high-performance liquid chromatographaic (HPLC) methods in which benomyl is determined as carbendazim seem to be very popular (Austin and Briggs, 1976; Kirkland, 1973; Kirkland et al., 1973). In one particular case, carbendazim, present as a natural degradation compound in a sample, was converted to and determined as benomyl; in this case the benomyl, which existed in the sample in its intact form from the beginning, was also determined as benomyl (Zweig and Gao, 1983). Results obtained with either of these conversion methods, however, do not represent the biological activity of samples. Carbendazim, which is deliberately produced from benomyl during the analytical procedure, cannot be distinguished from carbendazim, which was present in the sample as a natural degradation compound of benomyl. Similarly, benomyl, converted from carbendazim, cannot be distinguished from benomyl which was present in its intact form from the beginning.

Although the above kind of approach is not generally acceptable in pesticide residue methodologies, in the case of benomyl, this approach was well accepted in the past, mainly because carbendazim is also fungitoxic and because analysis of intact benomyl is difficult. The fungitoxicity of carbendazim, however, is markedly different from that of benomyl (Hall, 1980; Koller et al., 1982). Accordingly, the total quantity expressed as carbendazim or benomyl cannot be used to assess the toxicity or biological activity of fungicides present in a test sample.

Research Station, Agriculture Canada, Vineland Station, Ontario L0R 2E0 (M.C.), and Chemistry Department, Brock University, St. Catharines, Ontario L2S 3A1, Canada (R.P.S.).

[1]Supported by a grant (Grant No. G0780) from the Natural Sciences and Engineering Research Council of Canada (NSERC). Present address: Department of Chemistry, State University of New York, Buffalo, NY 14214.

0021-8561/86/1434-0108$01.50/0