Food Chem. 22, 1015–1019 (1974).
Mirocha, C. J., Christensen, C. M., Nelson, G. H., *Microb. Toxins* **7**, 103–138 (1971).
Pathre, S. V., Mirocha, C. J., *Adv. Chem. Ser.*, in press (1976).
Steele, J. A., Ph.D. Thesis, University of Minnesota, 1974.
Steele, J. A., Lieberman, J. R., Mirocha, C. J., *Can. J. Microbiol.* **20**, 531–534 (1974).
Wolf, J. C., Mirocha, C. J., *Can. J. Microbiol.* **19**, 725–734 (1973).

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Monoacetoxyscirpenol. A New Mycotoxin Produced by *Fusarium roseum* Gibbosum

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Fusarium roseum Gibbosum was isolated from corn suspected of causing illness and death in several dairy cattle. When this isolate was grown on corn or rice and consumed by rats, turkey poults, swine, or young chickens, it caused illness and death. Two toxic metabolites were isolated from corn on which this isolate was grown. Chemical and spectral evidence indicate that both toxins are 12,13-epoxytrichothecenes. Monoacetoxyscirpenol, a major toxic component, has been shown to be 15-acetoxy,-3α,4β-dihydroxy-12,13-epoxytrichothec-9-ene and is the only toxic component in the culture when grown on rice. The other toxic component is scirpentriol (3α,4β,15-trihydroxy-12,13-epoxytrichothec-9-ene), found when grown on corn. Monoacetoxyscirpenol is the principal toxic metabolite produced by this isolate of *Fusarium*.

In Minnesota, a number of undiagnosed cases of animal toxicosis pass through the Veterinary Diagnostic Laboratory which have no explanation except that perhaps the feed might be implicated in explaining signs of intoxication such as abortion, diarrhea, emesis, loss of weight gain, hemorrhagia, and death. Routinely, these feed samples are analyzed for toxic fungi and toxic components. One such case involved abortion and death in dairy cattle and swine. An isolate of *Fusarium roseum* Gibbosum, among other fungi, was isolated from the feed, grown on autoclaved corn, and analyzed for toxicity in rats. One major toxic metabolite was isolated from this isolate and its identification is the subject of this paper.

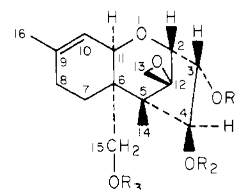
RESULTS AND DISCUSSION

The cultures of *Fusarium roseum* Gibbosum grown on rice were harvested, dried, moistened, and extracted with ethyl acetate (see Scheme I for extraction and cleanup procedure). Toxicities of the various fractions were determined by feeding to white rats.

The extensive chromatographic separations of the partially purified extract resulted in the isolation of a pure crystalline toxin. Its molecular weight was determined by high-resolution mass spectroscopy and found to be 324, with an elemental analysis of $C_{17}H_{24}O_6$. Preliminary interpretation of the mass spectral data showed an m/e^+ of 264 (measured 264.1373) as the highest significant mass in the spectrum with an elemental composition of $C_{15}H_{20}O_4$. However, the mass spectrum of the trimethylsilyl (Me_3Si) ether showed an apparent molecular ion at m/e^+ 468 (measured 468.2387 and elemental composition of $C_{23}H_{40}O_6Si_2$), instead of the expected molecular ion at m/e^+ 408 ($C_{21}H_{36}O_4Si_2$) which was due to the loss of acetic acid from the parent ion (m/e^+ 468). Therefore, the ion at m/e^+ 264 in the underivatized toxin was not the molecular ion, but rather an ion arising from the rapid loss of CH_3COOH from the parent ion (Figure 1).

The infrared spectrum (Figure 2) of the toxin in a KBr disk showed the presence of a free hydroxyl (3400 cm^{-1}), an ester group (1715, 1250 cm^{-1}), and an olefinic linkage (3010, 1680, 835 cm^{-1}). The ultraviolet spectrum did not show any significant absorption except end absorption. Deuterium exchange, trimethylsilylation, and acetylation indicated the presence of two hydroxyl groups.

The toxin yielded a triol **3** when hydrolyzed in a solution of 0.6 *N* K_2CO_3 which when acetylated by *N*-acetylimidazole in refluxing methylene chloride was quantitatively converted into its triacetate **4**. This reaction proceeded smoothly and was much simpler than the classical acetylation by acetic anhydride; see structures **1–8** of



	R_1	R_2	R_3
1	H	H	$COCH_3$
2	H	$COCH_3$	$COCH_3$
3	H	H	H
4	$COCH_3$	$COCH_3$	$COCH_3$
5	Me_3Si	Me_3Si	$COCH_3$
6	$COCF_3$	$COCF_3$	$COCH_3$
7	D	D	$COCH_3$
8	H	$COCH_3$	H

various derivatives of the trichothecene toxins.

The triacetate **4** formed in the above reaction was identical with the acetylation product of the toxin. Hydrolysis of **4** in 0.2 *N* NH_4OH–MeOH yielded a mixture consisting of the triol **3**, the toxin, and a monohydroxy compound. The latter was identified as diacetoxyscirpenol **2** (3α-hydroxy,4β,15-diacetoxy-12,13-epoxytrichothec-9-ene), a known mycotoxin (Sigg et al., 1965; Dawkins, 1966;

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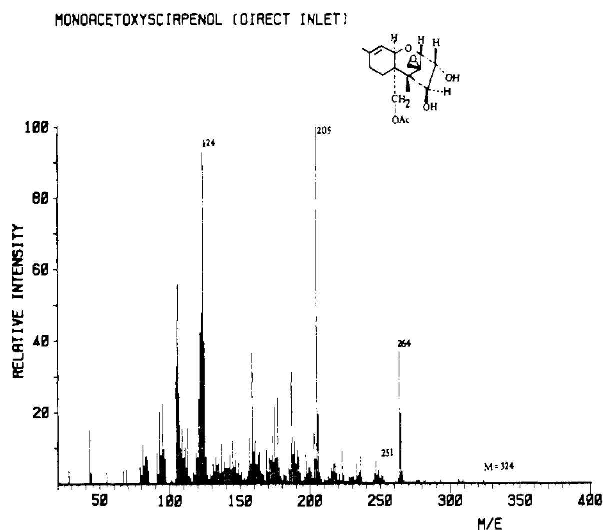


Figure 1. Mass spectral analysis of monoacetoxyscirpenol by the direct probe method.

Bamburg et al., 1968). Reduction of the toxin and its triol derivative with $LiAlH_4$ in refluxing tetrahydrofuran yielded the identical tetraol (Sigg et al., 1965; Dawkins, 1966). Thus, the triol was identified as $3\alpha,4\beta$,15-trihydroxy-12,13-epoxytrichothec-9-ene. Further, diacetoxyscirpenol, upon partial hydrolysis in a solution of methanolic ammonia, gave a product identical with the toxin. Hence, the toxin is a monoacetate of **3** (monoacetoxyscirpenol) and has either structure **1** or **8**. When solvolysis of **2** was carried out in aqueous methanolic sodium acetate (pH 9.5), the product isolated was almost exclusively monoacetoxyscirpenol. We attributed such solvolysis under these mild conditions to the combined effect of neighboring group participation (Grove, 1970) and intramolecular hydrogen bonding of the C-3 hydroxyl group to produce the 3,4-diol **1** from diacetoxyscirpenol **2** (Scheme II). Analogous results were reported by Wei et al. (1971) in the mild alkaline hydrolysis of 3α-hydroxy-4β,15-diacetoxy-8α-isovaleroxy-12,13-epoxytrichothec-9-ene (T-2 toxin).

Unambiguous assignment of the position of the acetate group was made from analysis of the NMR spectral data shown in Table I and Figure 3. The general appearance of the spectrum is indicative of a 12,13-epoxytrichothec-9-ene skeleton. The parent ring is defined in general by the AB quartet at δ 2.76 and 3.04 (J = 3.8 Hz) due to the methylene protons at C-13, a singlet (3 H) at δ 0.8 due to the methyl group at C-5, a broad singlet (3 H) at δ 1.73 of the methyl group at C-9, and a clean doublet centered at δ 3.63 (J = 5 Hz) due to the C-2 proton. The NMR spectra of monoacetoxyscirpenol and scirpentriol are similar except that the former has one acetyl signal, two exchangeable protons, and an AB quartet of C-15 methylene protons centered at δ 4.05 (J = 12 Hz). This AB

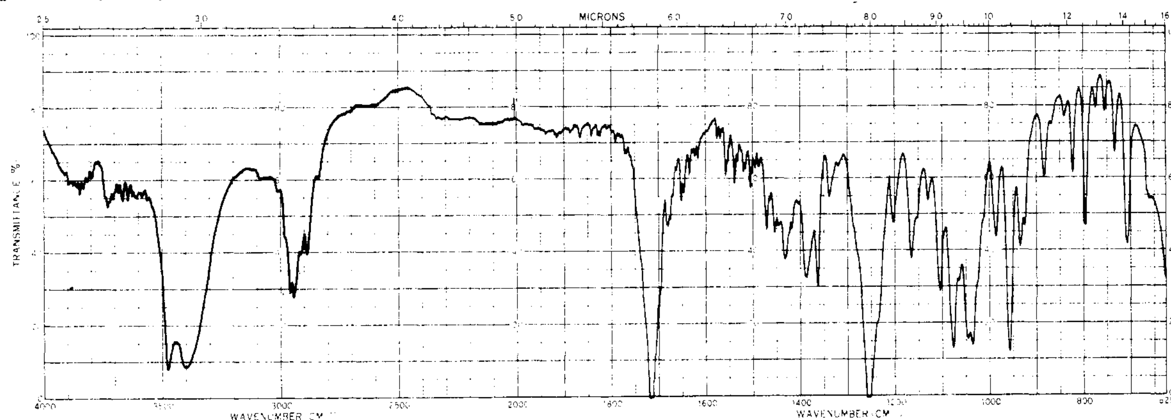


Figure 2. Infrared spectrum of monoacetoxyscirpenol.

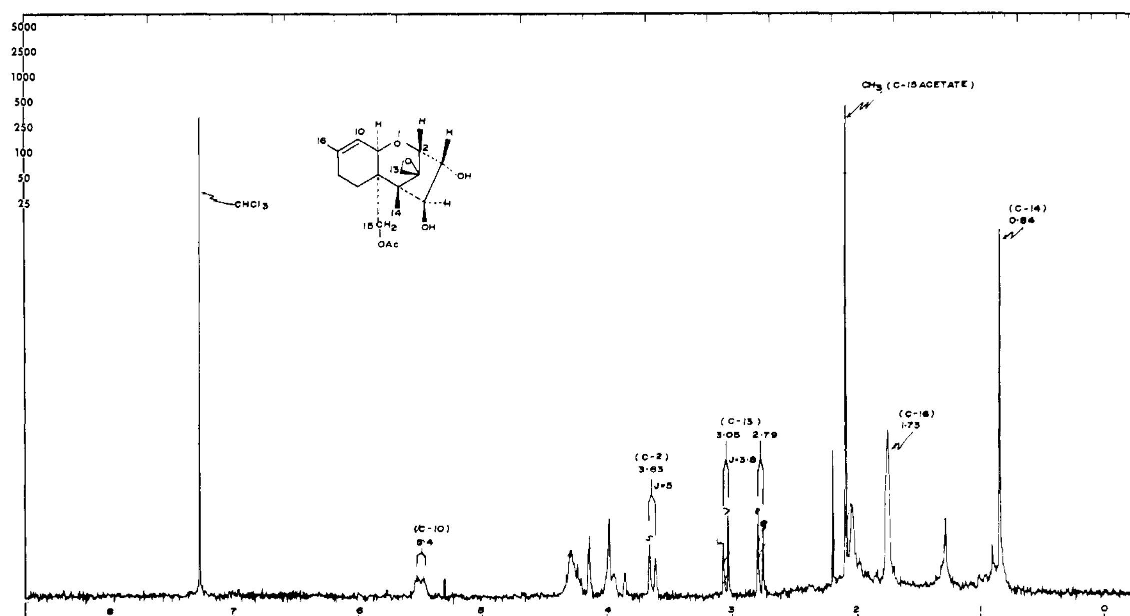


Figure 3. Analysis of monoacetoxyscirpenol by NMR.

Scheme I. Extraction Scheme Used in Recovering Monoacetoxyscirpenol from Culture

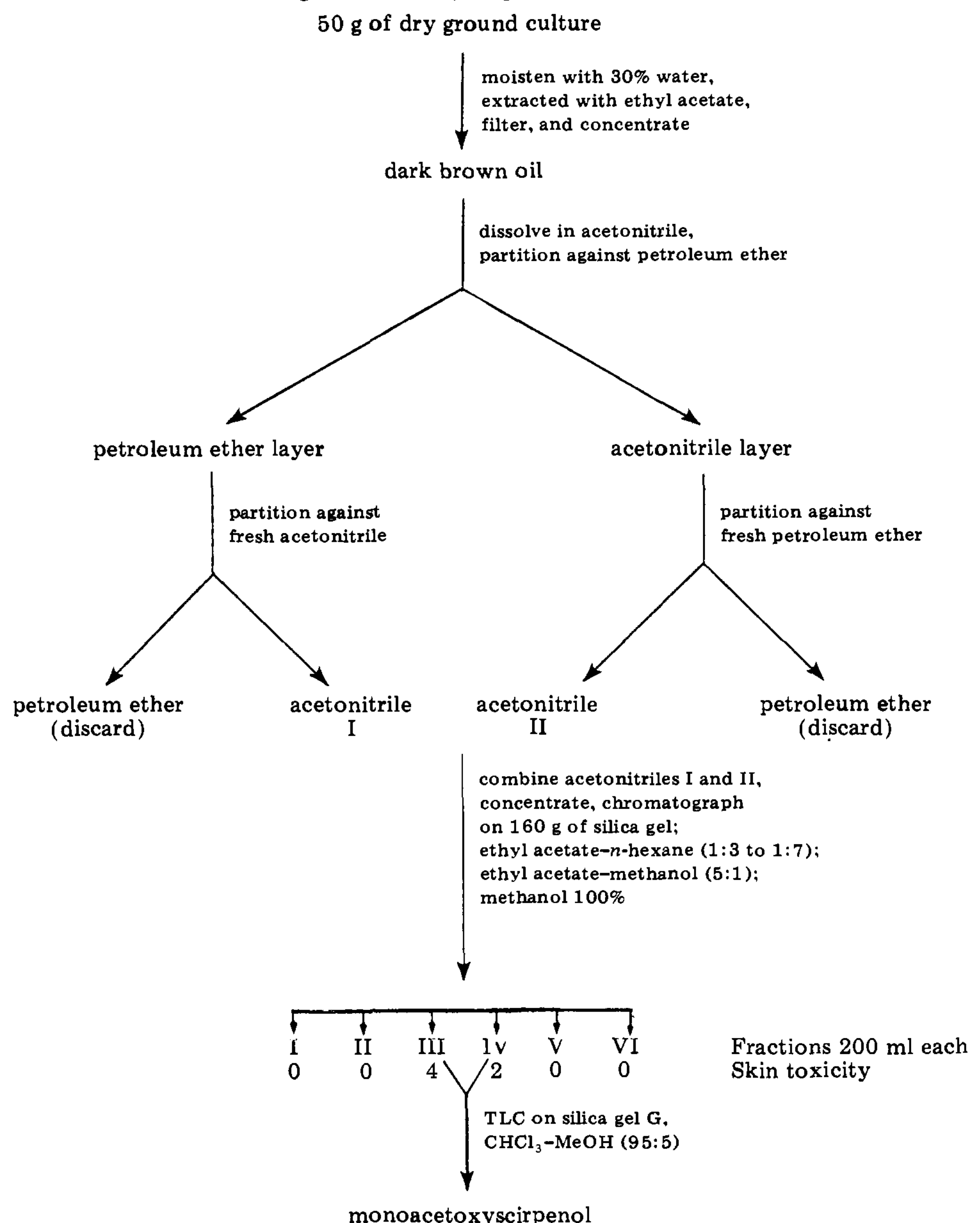


Table I. Chemical Shifts of Protons (ppm) in the ^{1}H NMR Spectra of Monoacetoxyscirpenol and Its Derivatives[a]

Compd	Positions						
	2	3	4	13	14	15	16
1	3.63 d (5)	4.22 dd (5, 2.8)	4.31 d (2.8)	2.79 d, 3.04 d (3.8) (3.8)	0.83 s	4.05 q (12)	1.73 br s
2	3.68 d (5)	4.12 dd (5, 2.8)	5.16 d (2.8)	2.79 d, 3.05 d (4) (4)	0.84 s	4.13 q (12)	1.74 s
3[b]	3.78 d (4.5)	4.08 dd (4.5, 2.8)	4.12 d (2.8)	2.70 d, 3.05 d (4) (4)	0.90 s	3.59 q (12)	1.72 br s
4	3.87 d (5)	5.20 dd (5, 3.3)	5.77 d (3.3)	2.81, 3.08 d (4) (4)	0.78 s	4.16 q (12)	1.73 br s

[a] Coupling constants are given in hertz (J). [b] The spectrum was not well resolved; however, addition of DCl considerably improved the resolution; d = doublet; dd = doublet of doublets; s = singlet; q = quartet; br s = broad singlet.

quartet (2 H) in **3** is centered at δ 3.67 (J = 12 Hz). The downfield shift of the quartet in monoacetoxyscirpenol is apparently due to acetylation of the C-15 hydroxyl of **3**. A multiplet observed around δ 4.2 in the former is due to protons at C-3 and C-4 as evidenced by decoupling experiments.

Upon irradiation of the resonances at δ 4.3, the multiplet at δ 4.3 collapses to a doublet centered at δ 4.2, J = 5 Hz (which was the A portion of the AB system consisting of protons at C-2 and C-3), and a singlet which was a part of the AB quartet due to C-15 protons. A rather ill-defined quartet at δ 4.1 was observed when the resonance at δ 3.6 was irradiated (J = 2.8 Hz). The NMR spectrum of **2** is different from that of the toxin only with respect to the C-4 proton.

Mass spectral studies of a number of derivatives of the toxin (**4** through **7**) were made. Interpretation (Bamburg and Strong, 1971) of the high-resolution mass spectra of

Scheme II. A Possible Transition State in the Formation of Monoacetoxyscirpenol from Diacetoxyscirpenol (The Hydrogen Bonding Facilitates the Hydroxide Attack)

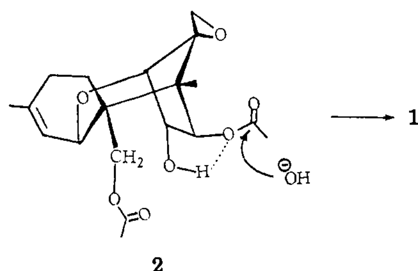


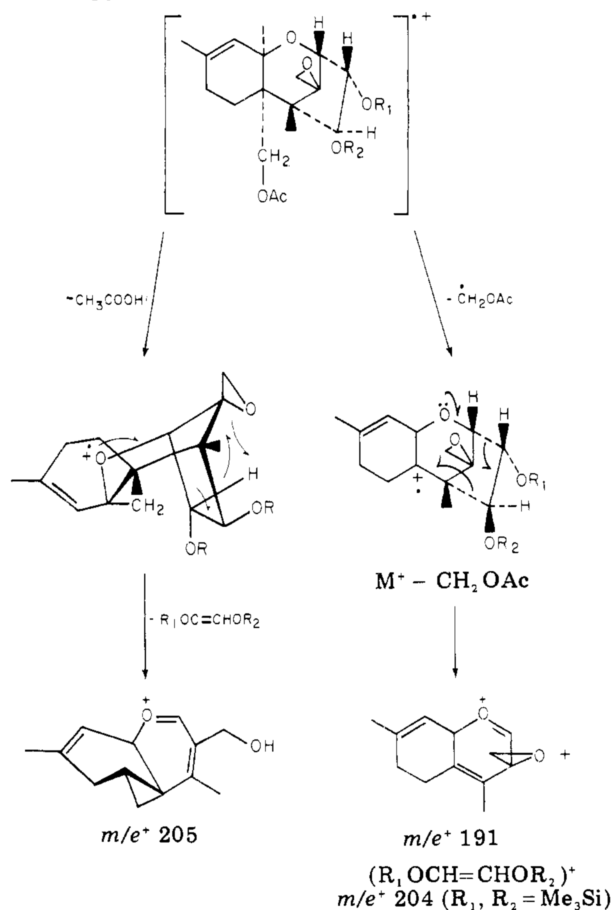
Table II. Effect on Turkey Poults of a Nutritionally Balanced Diet Amended with Different Concentrations of a Culture of *Fusarium roseum* Gibbosum Grown on Rice[a]

Amendment with *Fusarium* culture, %	Estimated MAS consumed/bird, av,[b] mg	Ration consumed/bird, g	Av wt gain or loss per bird at termination, g	Signs
1.25	0.712	57	15.8	BLIB[c]
2.50	0.375	15	−23.2	BLIB
5.0	0.55	11	−33.6	BLIB, death by 7 days
Control	0.00	165	122.0	Normal

[a] Analyses of this culture determined that the sole toxic agent present in the culture was monoacetoxyscirpenol (MAS). [b] Average of five birds. [c] BLIB = bilateral inflammation of the beak. The duration of the experiment was 7 days.

these derivatives indicated (see Scheme III): (a) loss of $-CH_2OAc$ from the parent ion, (b) loss of $C_2HO_2D_2$ from the $M^+ - HOAc$ ion to give a fragment at m/e^+ 205 in the deuterated compound, and (c) a strong peak at m/e^+ 204 with the elemental composition of $C_8H_{20}O_2Si$ in the Me_3Si ether of the toxin. The above confirm the presence of a *vic*-glycol system and are consistent with the structure of monoacetoxyscirpenol being $3\alpha,4\beta$-dihydroxy,15-acetoxy-12,13-epoxytrichothec-9-ene. Loeffler et al. (1967) reported the isolation of a cytotoxic metabolite from a liquid culture of *Fusarium concolor* which they called monodeacetylanguidin, the deacetyl derivative of anguidin. Anguidin was found to be identical with diacetoxyscirpenol (Loeffler et al., 1965). Since the position of the acetyl group of monodeacetylanguidin was not established, Bamburg and Strong (1971) referred to this metabolite as 4- or 15-acetylscirpentriol.

Scheme III. Fragmentation Pattern of Monoacetoxyscirpenol When Analyzed by Mass Spectroscopy



BIOLOGICAL ACTIVITY

The toxicity of monoacetoxyscirpenol was determined using turkey poults, laying chicken hens, rats, and swine. The toxicity was found, in general, to be similar to that of diacetoxyscirpenol in that monoacetoxyscirpenol produced bilateral inflammation of the beak area of birds ingesting the diet and also caused gastrointestinal hemorrhaging. It differed from the T-2 toxin (Bamburg et al., 1968) [3-hydroxy-4,15-diacetoxy-8-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene] in that the latter did not produce hemorrhaging, but was similar in that both caused bilateral inflammation of the beak.

When a rice culture of *Fusarium roseum* Gibbosum was added to an otherwise nutritionally balanced diet and fed to turkey poults, a bilateral inflammation of the beak developed until some birds were not able to feed. At higher concentrations, the turkey poults died within 7 days (Table II). When this same material was fed to laying hens, an immediate loss in weight gain and egg production was noted (Table III).

When the extract from the culture of *F. roseum* Gibbosum (equivalent to 1 g dry weight) was applied to the shaved skin of the rat, hyperkeratosis, petechial hemor-

Table III. Body Weight, Feed Consumption, and Egg Production of White Leghorn Hens Fed a Nutritionally Balanced Diet Amended with a Culture of *F. roseum* Gibbosum Grown on Corn Containing Monoacetoxyscirpenol (MAS) as the Sole Toxic Agent

Treatment	Total MAS consumed, mg	Body wt, g		Feed consumption, g/day		Egg production, eggs/week	
		Preexptl[a]	Exptl[b]	Preexptl	Exptl	Preexptl	Exptl
2.5% *F. roseum* Gibbosum	0.55	1523	1236	98	22	5.83	0.54
5.0% *F. roseum* Gibbosum	0.40	1570	1137	97	8	5.83	0.46
Control	0.00	1558	1576	97	96	6.08	5.96

[a] Preexperimental period of 14 days and experimental period of 28 days. [b] Each treatment mean represents data from six hens.

rhaging and a severe gastrointestinal hemorrhaging resulted. The LD_{50} in 20-day-old white female weanling rats (administered by subcutaneous injection) was found to be 0.752 ± 0.029 mg/kg body weight. A single female pig weighing 20 kg (prepubertal gilt) was injected intravenously with 20 mg of monoacetoxyscirpenol (1 mg/kg) and observed. Administration of toxin caused emesis within 0.5 hr, lethargy, a staggered gait, and death within 13 hr.

BIOSYNTHESIS OF MONOACETOXYSCIRPENOL AND OTHER METABOLITES

The isolate of *F. roseum* Gibbosum used in this study, when grown on autoclaved solid rice culture, produced approximately 1000 ppm (1 g/kg) of monoacetoxyscirpenol and ca. 12000 ppm (12 g/kg) of zearalenone [6-(10-hydroxy-1-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone] (Mirocha and Christensen, 1974). When grown on a solid corn grits medium, monoacetoxyscirpenol, scirpenetriol 3, and a small amount of zearalenone were produced, but in neither the rice nor the corn cultures was diacetoxyscirpenol produced; the latter was produced, however, in a liquid Czapek's medium. In order to detect possible artifacts due to the extraction procedure, diacetoxyscirpenol was added exogenously to the uninoculated rice and corn cultures and extracted with the same solvent system and sequence as monoacetoxyscirpenol. Only the added diacetoxyscirpenol was recovered (ca. 80% yield), illustrating that the former metabolite was not an artifact introduced by partial hydrolysis of the latter during extraction procedures.

Our past experience with *Fusarium* toxicosis lead us to the conclusion that *Fusarium roseum* and not *F. tricinctum* is most commonly associated with corn implicated in field cases of toxicosis. The latter species is most commonly associated with the production of the T-2 toxin and thought to be the primary incitant of the moldy corn disease (Hsu et al., 1972). In our experience, *F. roseum* is toxic to rats, in addition to causing the hyperestrogenic syndrome in animals, and in all probability is toxic by virtue of the production of the scirpene type of trichothecenes such as mono- and diacetoxyscirpenol. Based on the above evidence, we believe that *F. roseum* is more important in terms of frequency of occurrence in the production of the moldy corn diseases than the T-2 toxin as described by Smalley et al. (1970).

ANALYSIS OF MONOACETOXYSCIRPENOL

The above metabolite can be extracted from fungus cultures and mixed feed samples with ethyl acetate followed by partitioning between petroleum ether and acetonitrile (described in the experimental section). The toxin is then resolved by either TLC or GLC, or both, depending on the purity of the preparation. Monoacetoxyscirpenol has an R_f value of 0.37 on a silica gel plate when developed in $CHCl_3$–MeOH (90:10, v/v); it is made visible on the plate by either spraying with concentrated H_2SO_4 or *p*-anisaldehyde followed by heating at 110°C for 10 min. The toxin turns purple to brown when charred with H_2SO_4 and purple with *p*-anisaldehyde reagent. Monoacetoxyscirpenol can be quantitatively converted into the bis(trimethylsilyl) ether, diacetate, or bis(trifluoroacetate) for analysis by gas–liquid chromatography. A typical analysis by GLC of these derivatives is shown in Figure 4. Confirmation of identity is made by combined gas chromatography–mass spectrometry, and in addition this technique offers the advantage of separation and identification when the components appear in a mixture.

EXPERIMENTAL SECTION

General Procedures and Instrumentation. Drying of solution extracts, unless stated otherwise, was done with

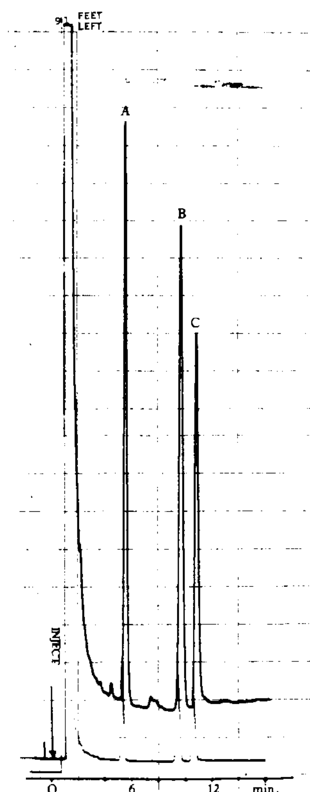


Figure 4. Separation of various derivatives of monoacetoxyscirpenol (MAS) by gas–liquid chromatography: (A) 3,4-bis(trifluoroacetate) derivative of MAS; (B) bis(trimethylsilyl) derivative of MAS; (C) diacetoxy derivative of MAS.

anhydrous magnesium sulfate and concentration was done under reduced pressure using a rotary evaporator. Infrared spectra were determined on a Perkin-Elmer 257 recording spectrophotometer in a KBr disk. Nuclear magnetic resonance spectra were determined in deuteriochloroform on a Varian Associates XLFT-100. The proton signal from residual [^{3}H]chloroform was used as an internal standard, and chemical shifts were then calculated as downfield from tetramethylsilane. Ultraviolet spectra were determined in methanol using a Beckman DBDG recording spectrophotometer. Gas chromatographic analyses were carried out on a Varian Aerograph 1500 equipped with flame ionization detector. A stainless steel column (3 ft × $^1/_8$ in. o.d.) packed with 3% OV-1 on Gas-Chrom Q of 100–120 mesh was used throughout the analyses. Other GC parameters were: column temperature programmed from 150 to 275°C at 6°C/min and carrier gas (N_2) and hydrogen flow rates were 20 ml/min. Mass spectra were determined at 70 eV using a LKB-9000 combination gas chromatograph–mass spectrometer. High-resolution mass spectra were obtained with an AEI-MS 30 double focusing mass spectrometer using an internal standard of perfluorokerosene at a resolution of 10000 and scan speed of 30 sec/decade. Masses were calculated with an on-line computer. Thin-layer chromatograms were run on Brinkmann silica gel G. Melting points were determined on a "melttemp" apparatus and are uncorrected for error. Elemental analyses were performed by the Clark Analytical Co., Inc., of Urbana, Ill.

Growth of Fungus Cultures and Determination of Toxicity. The isolate of *Fusarium roseum* Gibbosum used in these studies was grown on autoclaved, dehulled Uncle Ben's parboiled rice with a moisture content of 40% (fresh weight basis) after autoclaving. The seeded rice culture medium was kept at 25°C for 7 days and for 21 days at

12°C. At the end of this period, they were dried and ground to a fine mesh, after which they were either extracted or fed to rats for determination of toxicity. The ground cultures, when fed to rats, were incorporated into a nutritionally balanced diet at 50% of the toxicological diet; the diet was that as determined by Wogan and Newberne (1967). The rats used were 20-day-old white weanling females purchased from the Hotzman Company (Madison, Wis.).

Toxic extracts were also assayed on rats for determination of toxicity; 100 g of the culture was extracted with 500 ml of ethyl acetate, concentrated to 50 ml, and incorporated in the toxicological diet as described before.

Toxicity was also determined by topical application of the extract to the shaved skin of rats used in these studies. An area (2 × 3 cm) was shaved on the back of the rat, and 100 μl of the test solution applied to the skin each day for 5 days. The skin reactions were graded on a scale of 0 to 5 as set up by Bamburg (1969) as follows: (0) no observable reaction; (1) slight reaction followed by the formation of a light scab; (2) appreciable edema or inflammation over the inoculated area; (3) severe edema, together with spreading of the affected area; heavy scab formation; (4) marked petechial hemorrhaging in the affected area; (5) death of the animal.

Production and Isolation of Monoacetoxyscirpenol 1. The dried cultures, prepared as described before, were moistened with water to a moisture content of 30% and 500-g portions were extracted with ethyl acetate. The extract was concentrated to a gum and redissolved in 150 ml of acetonitrile and partitioned against 150 ml of petroleum ether (bp 60–70°C); the petroleum ether layer was discarded. The acetonitrile layer was concentrated to 20 ml and then chromatographed on a column of 700 g of silica gel. The components were eluted off the column with solvents depicted in Scheme I and collected into 100-ml fractions. Each fraction was tested for toxicity by topical application to the shaved skin of the white rat. Fractions 4 through 12 (100 ml) were toxic and were pooled and rechromatographed on a column of 80 g of silica gel using chloroform–methanol (98:2, v/v) as the eluting solvent. The fractions from this column were tested for toxicity and the one found toxic was purified by preparative TLC on silica gel G (20 mm × 20 mm), developed in chloroform–methanol (98:5) and made visible by charring with concentrated H_2SO_4. The toxic component so purified was crystallized from methylene chloride–petroleum ether (yield 78 mg). This compound had a wide melting point, 75–81°C. Two recrystallizations from isooctane–ethyl acetate gave 50 mg of an analytical sample: mp 172–173°C; ir (KBr) 3400, 1715, 1250 cm^{-1}; NMR ($CHCl_3$) δ 2.7 (d, 1, J = 4 Hz), 3.04 (d, 1, J = 4 Hz), 2.16 (s, 3, $-OOCCH_3$), 1.73 (br s, 3, $=CCH_3$), 0.83 (s, 3, $-CH_3$); mass spectrum m/e^+ (relative intensity) 264 (36.61), 205 (100), 191 (9.37), 189 (11.83), 187 (30.92), 177 (24). Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.96; H, 7.41. Found: C, 63.42; H, 7.61.

Analysis by Combination Gas Chromatography–Mass Spectrometry (GC–MS). Low-resolution mass spectra were recorded on a LKB-9000 GC–MS. A sample of the test solution (1 μl) was injected into the instrument, and mass spectral scans (4–6 sec) were taken of each component in question at the apex of the peak. The background spectra were obtained immediately before and after the appearance of the desired peak, and multiple scans were taken of each peak to ensure that only one component was present in each peak tested. All scans were taken at 70 eV.

Preparation of Derivatives for Analyses by High- and Low-Resolution Mass Spectrometry. (a) *Trimethylsilyl Ether* (*Me_3Si Ether*). A Me_3Si ether derivative of the material in question was prepared by allowing 20 μg of the latter to react with 20 μl of Tri-Sil-BT (Pierce Chemical Co., Rockford, Ill.) in a small reaction vessel (Reactivial). The reaction mixture was kept in the closed vial at 60°C for 30 min and then analyzed directly by combination gas chromatography–mass spectroscopy (GC–MS). To obtain a high-resolution mass spectrum, 5 μl of the reaction mixture was transferred into a capillary tube, the excess solvent removed in vacuo, and the capillary inserted directly into the direct probe inlet of the mass spectrometer.

(b) *Trifluoroacetate Derivative*. *N*-Methylbis(trifluoroacetamide) (Pierce Chemical Co., Rockford, Ill.) was used as the acetylating agent in a manner similar to that for the preparation of the Me_3Si ether derivative. Analyses by GC–MS and high-resolution mass spectrometry were obtained as with the Me_3Si ether described above.

(c) *15-Acetoxy,3α-,4β-dideuteroxy-12,13-epoxytrichothec-9-ene*. A 2-μl aliquot of a slurry of 0.5 mg of 1 in 100 μl of D_2O (>98%) was directly introduced into the direct probe inlet of the high-resolution mass spectrometer; analyses so obtained indicated almost 100% exchange: mass spectrum (70 eV) m/e^+ (relative intensity) 266 (36.0), 205 (100), 264 (0.0).

Hydrolysis of Monoacetoxyscirpenol. One milliliter of monoacetoxyscirpenol in methanol (5 mg/ml) was added to 1 ml of K_2CO_3 (0.6 *N*) in aqueous methanol and the mixture held at room temperature for 6 hr. After completion of the reaction, the solvents were removed in vacuo. The reaction product was extracted twice with 5 ml of ethyl acetate and dried over anhydrous $MgSO_4$. The extract, after Me_3Si ether derivatization, was analyzed by GC–MS, and a single component was found: m/e^+ 498 (1.59), 408 (10.7), 395 (3.9), 277 (74.04), 103 (100). Removal of the solvent under vacuum gave a solid which was recrystallized from isooctane–ethyl acetate: mp 187–188°C; NMR indicated the absence of a methyl singlet due to acetate.

Acetylation of 1. Two milligrams of 1 was added to a solution of *N*-acetylimidazole (6 mg) in 5 ml of methylene chloride, stirred continuously, and then refluxed for 30 min. The mixture was separated by TLC ($CHCl_3$–MeOH, 98:2, v/v) and yielded 2.1 mg (95%) of **4**. Acetylation of **2** and **3** by the above method yielded triacetate derivatives which were identical with respect to melting points (123–124°C), GC–MS analysis, and R_f values on TLC: NMR δ 2.81 (d, 1, J = 4 Hz), 3.08 (d, 1, J = 4 Hz), 2.16 (s, 3, C-15 acetate), 2.13 (s, 3, C-3 acetate), 2.07 (s, 3, C-4 acetate).

Acetylation of 12,13-epoxytrichothec-9-ene alcohols with *N*-acetylimidazole was found to be rapid, reliable, and direct; the reaction products could be analyzed directly by GLC without any prior extraction from the reaction mixture. Results of acetylation reactions are listed in Table IV.

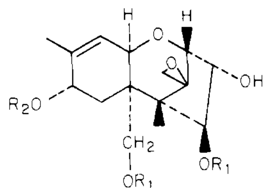
Hydrolysis of 4 in NH_4OH–MeOH. Three milligrams of **4** was added to a 2-ml solution of 0.2 *N* NH_4OH and the mixture was held at room temperature for 4 hr. The excess solvent was removed in vacuo and the reaction products resolved in TLC ($CHCl_3$–MeOH, 90:10), yielding 1.00 mg of **1**, 0.5 mg of **2**, and 1.2 mg of **3**. The NMR spectrum of **2** was similar to **1** and its identity as diacetoxyscirpenol confirmed by mass spectroscopy, NMR spectroscopy, and resolution by TLC using an authentic sample of diacetoxyscirpenol (Makor Chemicals Ltd., Jerusalem, Israel).

Solvolysis of 2 with Sodium Acetate. Three mil-

Table IV

Compound	Isolated, %	Analyses,[a] %
1	95	100
2	98	100
3	96	100
T-2 toxin[b]	96	100
T-2 tetraol[b]	96	100

[a] Determined by gas chromatographic analysis. [b] See structures in text.



T-2 toxin, $R_1 = CH_3CO-$; $R_2 = (CH_3)_2CHCH_2C(=O)$
T-2 tetraol, $R_1 = H$; $R_2 = H$

liliters of an aqueous methanolic solution of sodium acetate (10% w/v) was added to a solution of 5 mg of **2** in 2 ml of methanol. The pH of this mixture was brought to 9.5 by the addition of a drop of 0.01 *N* NaOH and the progress of solvolysis followed by TLC at 30-min intervals. At the end of 4 hr, the mixture was resolved on TLC ($CHCl_3$–MeOH, 90:10) and consisted of 75% of **1**, 20% of **2**, 3% of **3**, and 2% of an unidentified component (probably an epimer or an isomer of **2**).

LITERATURE CITED

Bamburg, J. R., Ph.D. Thesis, University of Wisconsin, Madison, Wis., 1969.
Bamburg, J. R., Riggs, N. V., Strong, F. M., *Tetrahedron* **24**, 3329–3336 (1968).
Bamburg, J. R., Strong, F. M., *Microb. Toxins*, Chapter 7 (1971).
Dawkins, A. W., *J. Chem. Soc.*, 116–123 (1966).
Grove, J. F., *J. Chem. Soc. C*, 375 (1970).
Hsu, I.-C., Smalley, E. B., Strong, F. M., Ribelin, W. E., *Appl. Microbiol.* **24**, 684–690 (1972).
Loeffler, W., Mauli, R., Rüsch, M. E., Stähelin, H., *Chem. Abstr.* **66**, 84744U (1967).
Loeffler, W., Mauli, R., Rüsch, M. E., Stähelin, H., *Chem. Abstr.* **62**, 5856*d* (1965).
Mirocha, C. J., Christensen, C. M., in "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, Amsterdam, 1974.
Sigg, H. P., Mauh, R., Flury, E., Hauser, D., *Helv. Chim. Acta* **48**, 962 (1965).
Smalley, E. B., Marasas, W. F. O., Strong, F. M., Bamburg, J. R., Nichols, R. E., Kosuri, N. R., *Proc. U.S.–Jpn. Conf. Toxic Micro-Org., 1st, 1968* (1970).
Wei, R.-D., Strong, F. M., Smalley, E. B., Schnoes, H. K., *Biochem. Biophys. Res. Commun.* **45**, 396 (1971).
Wogan, G. N., Newberne, P. M., *Cancer Res.* **27**, 2370 (1967).

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Characterization of Proteins and Allergens in Germinating Castor Seeds by Immunochemical Techniques

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Phosphate buffer extracts (pH 7.0–7.5) of mature ungerminated castor seeds, *Ricinus communis* L., contained at least seven major antigens detectable by immunoelectrophoretic analysis (IEA) against immune serum for the total castor seed proteins. The classical CB-1A allergen was identified in the total protein extract by using immune serum containing CB-1A antibodies. By IEA, specific proteins in certain allergen fractions were identified, and changes in the major proteins of dormant seed occurring after germination were easily detected. The CB-1A allergen contained a major antigen and at least two minor antigens. Some of the major proteins of dormant seeds changed significantly after 6 days' germination, but the major CB-1A antigen was still present.

Castor seed, *Ricinus communis* L., has long been an important industrial oilseed because of its unique and high oil content. It contains 50–60% oil and 18–20% protein. World production of the oil in 1971 was 325000 metric tons (Agricultural Statistics, 1972). The residue (over 100000 metric tons of defatted meal, or pomace) was generally used as fertilizer rather than as animal feed because it contained ricin (a toxalbumin), several potent allergens, and the alkaloid ricinine (Coulson et al., 1960; Waller and Negi, 1958). Attempts to detoxify castor seed meal have been reported (Mottola et al., 1972) but the products are still not considered major sources of protein for feed uses.

The allergens from defatted meals of mature castor seeds have been studied extensively. Berrens (1971) compared the electrophoretic migrations of 26 different allergens and concluded that atopic allergens were fast-moving anodic glycoproteins, but the basic allergenic proteins of castor seed were an exception to this general pattern. Layton et al. (1961) had examined the classical CB-1A allergen by paper electrophoresis and resolved it into six or more components at pH 8.0. Spies (1974) recently reviewed published research on allergens of dormant castor seeds, but few of the reports included the total proteins extracted from castor seeds.

Mourgue et al. (1958) separated the total proteins of

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