are known to be produced by several species of *Fusarium* such as *F. poae*, *F. tricinctum*, *F. sporotrichoides*, *F. roseum*, *F. avenaceum*, and *F. culmorum* (Ueno et al., 1972). This appears to be the first instance of a T-2 toxin isolated from a *Fusaria* belonging to the arthrosporiella group.

For the identification of trichothecenes, Ueno et al. (1973) have suggested a combination of biological tests using the rabbit reticulocyte assay and TLC analysis with H_2SO_4 acid spray. According to Smalley and Strong (1974), "Although this system has obvious uses in testing unknown pure culture for trichothecene production, it has not been tested on naturally contaminated food or feeds". However, in the present study, by employing the above methods in addition to the well-known skin irritant toxicity test, the presence of T-2 could be identified in a naturally contaminated food and in the fungus culture isolated from such food. Screening of samples of sorghum by this method for *Fusarium* toxicity is under progress.

The toxic metabolite found naturally in the moldy sorghum and the toxic metabolites obtained from rice infected with *F. incarnatum* appear to be identical. The natural occurrence of T-2 and a fungus responsible for producing T-2 toxin in a staple like sorghum acquires special significance in view of the reported relation of these types of toxins in causing human disease such as alimentary toxic aleukia reported from USSR (Joffe, 1974; Yagen and Joffe, 1976). Although population groups belonging to the poorer segments may be exposed to consumption of moldy sorghum, reports of a positive correlation with any disease attributable to this are so far lacking in India.

Besides T-2, several metabolites with fluorescent properties and other pigments were found in both the moldy sorghum and *Fusarium*-infected rice. However, the exact chemical nature and biological effect of these metabolites are not known and these aspects are under investigation currently. Long term feeding of moldy sorghum to rats and pups are also under way.

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LITERATURE CITED

Bamburg, J. R., Riggs, N. V., Strong, F. M., *Tetrahedron* **24**, 3329 (1968).
Ghosal, S., Chakrabarthy, D. K., Basu Chaudhury, K. C., *J. Pharm. Sci.* **65**, 160 (1976).
Jayaraman, S., Parihar, D. B., *Indian J. Exp. Biol.* **13**, 313 (1975).
Joffe, A. Z., "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, Amsterdam, 1974, p 229.
Sato, N., Ueno, Y., Enomoto, M., *Jpn. J. Pharmacol.* **25**, 263 (1975).
Saito, M., Ohtsubo, K., "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, Amsterdam, 1974, p 263.
Smalley, E. B., Marasus, W. F. O., Strong, F. M., Bamburg, J. R., Nichdo, R. E., Kosuri, N.R., Proceedings of the First U.S.-Japan Conference on Toxic Microorganism U.J.N.R., U.S. Department of Interior, Washington, D.C., 1970, p 163–173.
Smalley, E. B., Strong, F. M., "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, Amsterdam, 1974, p 199.
Subramanian, C. V., "Hypomycetes", an account of Indian species except *cercosporae*, Indian Council of Agricultural Research, New Delhi, 1971, p 713.
Ueno, Y., Ishikawa, Y., Amkai, K., Nakajima, M., Saito, M., Enomoto, M., Ohtsubo, K., *Jpn. J. Exp. Med.* **40**, 33 (1970).
Ueno, Y., Ishikawa, Y., Nakajima, M., Sakai, K., Ishii, K., Tsunoda, H., Saito, M., Enomoto, M., Ohtsubo, K., Umeda, M., *Jpn. J. Exp. Med.* **41**, 257 (1971).
Ueno, Y., Sato, N., Ishii, K., Sakai, K., Enomoto, M., *Jpn. J. Exp. Med.* **42**, 461 (1972).
Ueno, Y., Sato, N., Ishii, K., Sakai, K., Tsunoda, H., Enomoto, M., *Appl. Microbiol.* **25**, 699 (1973).
Ueno, Y., Shimada, N., Yagasaki, S., Enomoto, M., *Chem. Pharm. Bull.* **22**, 2830 (1974).
Yagen, G., Joffe, A. Z., *Appl. Environ. Microbiol.* **32**, 423 (1976).

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Two New Trichothecenes Produced by *Fusarium roseum*

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Two new toxic 12,13-epoxytrichothecenes were isolated from the culture filtrates of two isolates of *Fusarium roseum* and were characterized as 4-acetoxyscirpendiol and 8-acetylneosolaniol.

The 12,13-epoxytrichothecenes are a group of biologically active secondary metabolites predominantly associated with species of *Fusarium* but biosynthesized by many different fungi as well (Smalley and Strong, 1974). This group of toxins became well known after their implication in a disease of humans called alimentary toxic aleukia, described by Joffe (1971), and associated with cereal grains which overwintered in the field. Moldy corn toxicosis in farm animals as described by Hsu et al. (1972) in northern climates is associated with trichothecenes. *Fusarium roseum* is frequently isolated from moldy corn and feed commonly associated with corn implicated in field cases of mycotoxicoses (*Mirocha* and Christensen, 1974). During the course of screening some of these *Fusarium* isolates for trichothecene production, two strains of *Fusarium roseum* were found to produce heretofore uncharacterized trichothecenes. Their isolation and iden-

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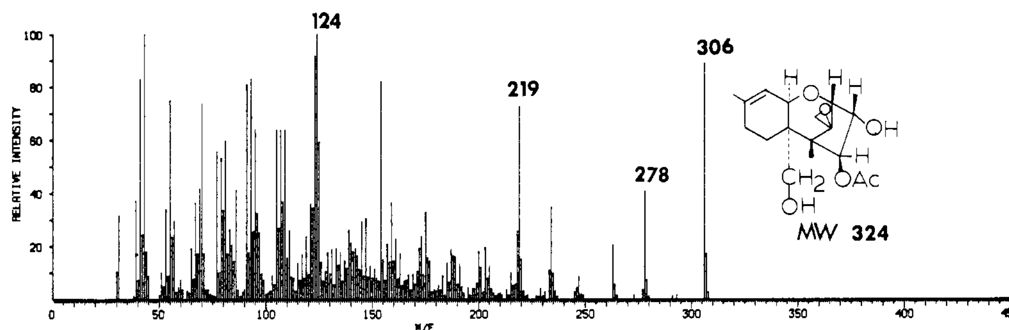
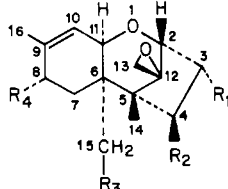


Figure 1. The mass spectrum of 4-acetoxyscirpendiol (70 eV).

Table I. Chemical Derivatives of Various Trichothecenes (OAc=CH_3COO-)



	R_1	R_2	R_3	R_4
1 4-Acetoxyscirpendiol	OH	OAc	OH	H
2 Monoacetylneosolaniol	OH	OAc	OAc	OAc
3 Monoacetoxyscirpenol	OH	OH	OAc	H
4 Diacetoxyscirpenol	OH	OAc	OAc	H
5 Scirpentriol	OH	OH	OH	H
6 Neosolaniol	OH	OAc	OAc	OH
7 T-2 tetraol	OH	OH	OH	OH
8 T-2 toxin	OH	OAc	OAc	$OCOCH_2$-$CH(CH_3)_2$
9 Diacetylneosolaniol	OAc	OAc	OAc	OAc

tification is the subject of this paper.

RESULTS AND DISCUSSION

4-Acetoxyscirpendiol. *Fusarium roseum* "Gibbosum" (3-8-66 No. 10), isolated from corn harvested in Minnesota, was grown on Czapek-peptone medium, and toxins were extracted from the culture filtrate according to the method previously reported (Ishii, 1975). The crude extract was fractionated by column chromatography on silica gel. Each fraction was tested for toxicity by the skin test using white rats (Bamburg, 1969). Four toxic compounds were isolated from these fractions. Three of the toxic compounds were identified by gas chromatography–mass spectrometry (GC–MS) as known trichothecenes: monoacetoxyscirpenol (**3**) (Pathre et al., 1976), diacetoxyscirpenol (**4**) (Sigg et al., 1965), and scirpentriol (**5**) (Table I).

The unknown toxic compound was also shown to be a trichothecene as it gave **5** when hydrolyzed with ammonium hydroxide. Acetylation of the unknown toxin yielded a triacetate identical with the acetylation product of **4**. The hydrolysis of **4** with 40% sulfuric acid (Sigg et al., 1965) gave a product identical with the unknown toxin. Sigg et al. (1965) reported the isolation of 4-acetoxyscirpendiol from the acidic hydrolysis of **4**. It is interesting to note that Loeffler et al. (1967) reported the isolation from a liquid culture of *Fusarium concolor*, of a cytotoxic metabolite which was described as monodeacetylanguidin, a compound similar to **1**. [Anguidin is identical with diacetoxyscirpenol (Loeffler et al., 1965).] Bamburg and Strong (1971) referred to monodeacetylanguidin as 4- or 15-acetylscirpentriol.

Unlike monoacetoxyscirpenol **3**, the mass spectrum of **1** (Figure 1) did not show the molecular ion at m/e^+ 324; however, a significant fragment at m/e^+ 306 due to the loss of H_2O from the molecular ion was observed. The mass spectrum of trimethylsilyl ether showed the molecular ion at m/e^+ 468.

The ^1H NMR spectrum of the new toxin showed the presence of one acetyl group (singlet, 3 H, at δ 2.10) in addition to the typical features of 12,13-epoxytrichothecenes (Bamburg and Strong, 1971). A typical AB quartet of the protons at C-15 was observed at δ 3.66 (J = 12 Hz). This quartet was shifted to δ 4.1 in **3**, **4** (Table II), and also in the acetylated product of the toxin. A multiplet (2 H) observed at δ 4.2 was assigned to the protons at C-3 and C-11. When this multiplet was irradiated, a doublet (1 H) at δ 5.51 collapsed to a singlet. These observations are consistent with the structure **1** of the unknown toxin: 4-acetoxyscirpendiol (4β-acetoxy-3α,15-dihydroxy-12,13-epoxytrichothec-9-ene).

8-Acetylneosolaniol. *Fusarium roseum* strain V-18, also isolated from corn harvested in Minnesota, was grown on Czapek-peptone medium. Chromatography of the crude extract of the culture filtrate yielded four toxic compounds. Three of them were known trichothecenes identified by TLC and GC–MS as diacetoxyscirpenol **4**, neosolaniol **6** (Ishii et al., 1971), and T-2 toxin **8** (Bamburg et al., 1968). The fourth toxic compound **2** was shown to be a monoacetate of **6** (8-acetylneosolaniol) by the following observations.

Hydrolysis of the unknown toxin gave T-2 tetraol **7** (Bamburg et al., 1968); acetylation yielded the acetate **9** identical with the acetylated products of **6** and **7**. The mass spectrum of **2** scanned at 70 eV (Figure 2) showed a rather weak (<1%) molecular ion at m/e^+ 424. Some major peaks observed were at m/e^+ 382 ($M^+ - CH_3CO$), 364 ($M^+ - CH_3COOH$), and 304 (364 − CH_3COOH). This pattern is highly indicative of the presence of at least two

Table II. Chemical Shifts of Protons (ppm) in the ^1H NMR Spectra of Related Trichothecenes[a]

Compound	Positions 2	3	4	7	8	15
1	3.66 d (5)	4.22 dd	5.51 d (3)			3.66 q (12)
2	3.80 d (5)	4.18 m	5.26 d (3)	2.30 d, 2.45 d	5.28 d	4.08 q (12)
3[b]	3.63 d (5)	4.22 dd	4.31 d (2.8)			4.05 q (12)
4[b]	3.68 d (5)	4.12 dd	5.16 d (2.8)			4.13 q (12)
9	3.86 d (5)	5.21 dd	5.86 d (3)	ca. 2.0, 2.4 dd	5.29 dd	4.21 q (12)

[a] Coupling constants are given in Hertz (J); d = doublet; s = singlet; q = quarter; m = multiplet. [b] Pathre et al., 1976.

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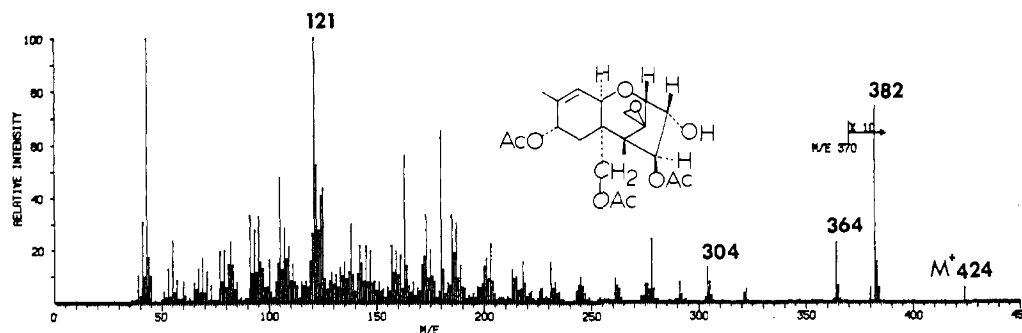

Figure 2. The mass spectrum of 8-acetylneosolaniol.

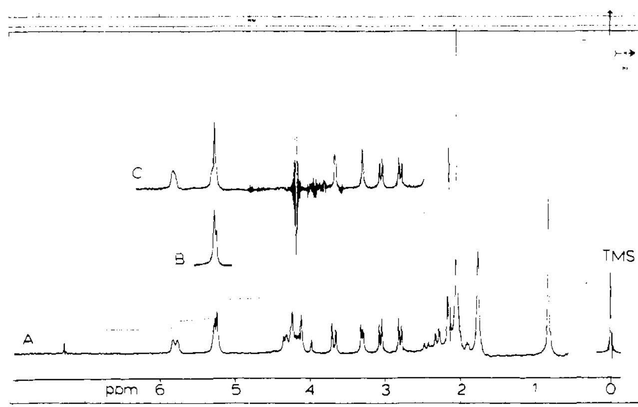

Figure 3. (A) Normal ^{1}H NMR spectrum of 8-acetylneosolaniol. (B) Partial collapse of the doublet at δ 5.2 by irradiation of the high field doublets at δ 2.3. (C) Decoupled spectrum with irradiation of the quartet at δ 4.2.

acetyl groups in the compound. The ^{1}H NMR spectrum of the toxin (Figure 3) shows the typical features of a 12,13-epoxytrichothecene nucleus (Bamburg and Strong, 1971). In addition, it displays singlets at δ 2.04 (6 H) and δ 2.14 (3 H), indicating the presence of three acetyl groups. Other notable features of the spectrum are doublets centered at δ 2.3 (1 H), 2.4 (1 H), 3.3 (1 H), and 5.26 (2 H), and a quartet overlapping a multiplet centered at δ 4.2. This quartet is a typical AB system for the protons at C-15. The chemical shift indicates that C-15 is acetylated. The multiplet at δ 4.2 is assigned to the protons at C-3 and C-11. When this multiplet was irradiated, the doublets at δ 3.3 (–OH at C-3), 3.8 (H at C-2), 5.2 (H at C-4), and 5.8 (H at C-10) were collapsed to broad singlets. The doublet at δ 3.3 disappeared when the chloroform-d_1 solution was shaken with DCl. Spin decoupling experiments (Figure 3B) also revealed that the doublet at δ 5.28, overlapping the doublet due to the proton at C-4, was the X part of an ABX system consisting of the protons at C-8 (X part) and C-7 (AB part).

The ^{13}C NMR spectrum of the toxin is shown in Figure 4. The spectrum was compared with those of neosolaniol and other trichothecenes reported by Ellison and Kotsonis (1976). The chemical shifts of compounds **2**, **6**, **7** and **8** are presented in Table III.

Three lowfield signals at δ 172.74, 170.82, and 170.33 were assigned to the acetate carbonyls at C-4, C-8, and C-15, respectively. The acetate carbonyl signal at δ 170.82 was absent in neosolaniol. The signal at δ 68.6 and 67.5 were assigned to C-8 and C-11, respectively, by comparison with the values previously reported for **7** and **8** (Ellison and Kotsonis, 1976). Acetylation of the hydroxyl at C-8 resulted in a characteristic downfield α shift for C-8 and upfield β shifts (Breitmaier and Voelter, 1974) for C-7 and C-9.

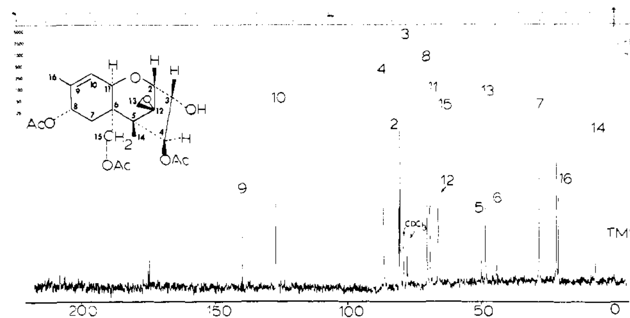

Figure 4. ^{13}C NMR spectrum of 8-acetylneosolaniol.

Table III. ^{13}C **Chemical Shifts (in ppm from** Me_4Si**) of the Trichothecenes**

	Compounds			
Positions	8-Acetyl-neosolaniol[a]	Neosol-aniol[b]	T-2 toxin[c]	T-2 tetraol[c]
C-2	78.9	78.5	78.7	78.5
C-3	78.4	78.9	76.0	79.1
C-4	84.6	84.8	82.4	80.0
C-5	48.7	48.8	48.2	48.0
C-6	43.1	43.5	42.2	45.1
C-7	27.4	30.4	26.7	28.5
C-8	68.6	67.8	67.4	64.8
C-9	136.5	139.8	134.6	138.1
C-10	124.0	121.3	124.2	121.6
C-11	67.5	66.8	66.3	67.6
C-12	64.4	65.0	64.1	64.3
C-13	47.2	47.3	46.2	45.1
C-14	7.0	6.8	6.4	6.3
C-15	64.5	65.0	63.9	61.0
C-16	20.3	20.5	19.4	19.9

[a] Three acetate methyls: 21.1 ppm; three acetate carbonyls: C-4, 172.7; C-8, 170.8 and C-15, 170.3 ppm. [b] Two acetate methyls: 21.0 ppm; two acetate carbonyls: C-4, 172.7 and C-15, 170.3 ppm. [c] Ellison and Kotsonis, 1976.

Finally, the hydrolysis of the tetracetate **9** in aqueous methanolic sodium acetate (pH 9.5) (Pathre et al., 1976) gave a product identical with the unknown toxin.

The foregoing evidence is consistent with the structure **2** of the unknown toxin, 8-acetylneosolaniol (4β,8α,15-triacetoxy-3α-hydroxy-12,13-epoxytrichothec-9-ene).

EXPERIMENTAL SECTION

Melting points were determined on a Fischer-Jones Micro Melting Point Apparatus and were uncorrected. Infrared spectra were taken on KBr pellets with a Perkin-Elmer 257 recording spectrometer. NMR (^{1}H, ^{13}C) spectra were measured in $CDCl_3$ solution with Me_4Si as an internal standard on a Varian XLFT-100 spectrometer (100 MHz). Mass spectra were determined at 70 eV with a LKB-9000 GC–MS spectrometer. GC–MS analysis of

trimethylsilyl (Me_3Si) derivatives were also carried out on the LKB-9000 equipped with a glass column (0.3 × 120 cm) packed with 3% OV-1 on 100–120 mesh Gas-Chrom Q. Silica gel (grade H, Davison Commercial) was used for column chromatography and TLC was carried out on silica gel G. For preparative layer chromatography (PLC), plates, 0.5 mm in thickness, were used. Detection of spots on TLC plates was carried out by spraying with 50% H_2SO_4 and heating until charred.

Culture of Fungi. Inocula were grown at 25 °C on potato dextrose agar slants for 14 days.

Erlenmeyer flasks (1 L) each containing 330 mL of Czapek-peptone medium were seeded with conidia and mycelia and incubated for 14 days at 25 °C.

Isolation of 4-Acetoxyscirpediol. (a) *F. roseum* "Gibbosum" (3-8-66 No. 10). Preparation of crude toxin from the culture filtrate (8.3 L) of *F. roseum* "Gibbosum" was carried out with the method previously reported (Ishii, 1975). The crude toxin (1.3 g) was loaded on a column (2.3 × 60 cm) packed with silica gel and eluted with benzene–acetone (2:1, 900 mL; 3:2, 500 mL; 1:1, 400 mL), acetone (400 mL), and methanol (400 mL). The eluate was monitored by TLC and divided into eight fractions (A 113, B 64, C 81, D 48, E 35, F 30, G 59, H 472 mg) according to R_f values of the components.

Fraction B was further purified by PLC with hexane–acetone (1:1). The purified material **1** was obtained as an amorphous solid (15 mg) from benzene–hexane, mp 100–110 °C. Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 60.93; H, 6.65. IR 3450, 2950, 1720, 1435, 1375, 1240, 1165, 1110, 1080, 1050, 960 cm^{-1}; MS m/e^+ 306, 278, 234, 219, and 43.

Parts of fraction A, D, and F were derivatized with Tri-Sil TBT (Pierce Chemical Co.) and analyzed with GC–MS as Me_3Si ethers. The GS–MS analysis revealed the presence of diacetoxyscirpenol, monoacetoxyscirpenol, and scirpentriol, respectively.

Isolation of 8-Acetylneosolaniol. Crude toxin (3.4 g) was obtained from the culture filtrate of *F. roseum* V-18 (6.6 L). The crude toxin was chromatographed on a silica gel column (2.3 × 60 cm) with *n*-hexane–acetone (2:1, 1350 mL; 3:2, 750 mL; 1:1, 1000 mL), followed by acetone (400 mL) and methanol (400 mL). The eluate was divided into eight fractions (I 79, II 216, III 392, IV 26, V 111, VI 225, VII 476, VIII 1516 mg). Fraction III was rechromatographed on a silica gel column (1 × 54 cm) with chloroform–acetone (7:1, 240 mL; 5:1, 120 mL; 3:1, 120 mL) and five fractions (III-1 10, III-2 230, III-3 40, III-4 30, III-5 43 mg) were obtained. Fraction III-2, III-3, and III-4 were combined and further purified by PLC (chloroform acetone, 7:1) to obtain **2**. The compound was recrystallized twice from benzene–hexane, mp 189–190 °C. Anal. Calcd for $C_{21}H_{28}O_9$: C, 59.43; H, 6.65. Found: C, 59.64; H, 6.61; IR bands at 3380, 1735, 1255, 1235, 1085, 1035, 970, and 960 cm^{-1}; MS m/e 424, 382, 364, 304, and 43.

Fractions I, II, and V were analyzed by GC–MS as their Me_3Si ethers. The analysis showed the presence of T-2 toxin, diacetoxyscirpenol, and neosolaniol, respectively, in fractions I, II, and V. Further purification of the fractions yielded 208 mg of diacetoxyscirpenol and 16 mg of neosolaniol.

Acetylation of Diacetoxyscirpenol. A solution of diacetoxyscirpenol (16 mg) in pyridine (0.5 mL) and acetic anhydride (0.5 mL) was kept at room temperature for 24 h, then diluted with cold water (5 mL) and extracted with ethyl acetate (5 mL × 2). The ethyl acetate extract was evaporated to an oil, which was applied on PLC and developed with *n*-hexane–acetone (1:1). The material at R_f 0.55 was crystallized from benzene–hexane to give 3α,4β,15-triacetoxy-12,13-epoxytrichothec-9-ene (10.5 mg), mp 123–124 °C (Calcd for $C_{21}H_{28}O_8$: C, 61.75; H, 6.91%. Found: C, 62.11; H, 6.84); IR bands at 1740, 1365, 1250, 1225, 1050, 1040, and 1030 cm^{-1}.

Acetylation of 1. A mixture of **1** (5 mg), pyridine (0.5 mL), and acetic anhydride (0.5 mL) was kept at room temperature for 24 h, diluted with water (5 mL), and extracted with ethyl acetate (5 mL × 2). The extract was subjected to PLC with *n*-hexane–acetone (1:1) and the purified material was crystallized from benzene–hexane as needles (4.5 mg), identical with 3α,4β,15-triacetoxy-12,13-epoxytrichothec-9-ene as determined by NMR, IR, and MS.

Hydrolysis of 1. Compound **1** (0.2 mg) was dissolved in 4 N NH_4OH–MeOH (0.3 mL) and kept at room temperature for 24 h. Part of the solution was evaporated in a vial under a stream of N_2. The residue was silylated and subjected to GC–MS. The MS of the main product was identical with that of Me_3Si ether of scirpentriol **5**.

Hydrolysis of Diacetoxyscirpenol with H_2SO_4. Diacetoxyscirpenol (1 mg) in 40% H_2SO_4 (2 mL) was kept at room temperature for 24 h, neutralized with 4 N NH_4OH, and extracted with ethyl acetate (5 mL × 3). The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was subjected to TLC with benzene–acetone (2:1) and the component corresponding to **1** (R_f 0.39) was isolated. The mass spectrum of the Me_3Si ether of this component was identical with the Me_3Si ether of **1**.

Acetylation of *Neosolaniol*. A mixture of neosolaniol (15 mg), acetic anhydride (1 mL), and pyridine (1 mL) was kept at room temperature for 20 h, diluted with 10 mL of H_2O, and extracted with ethyl acetate (5 mL × 2). The ethyl acetate solution was dried over anhydrous Na_2SO_4 and evaporated to dryness. The material was crystallized from benzene–hexane to give 3α,4β,8α,15-tetraacetoxy-12,13-epoxytrichothec-9-ene (neosolaniol diacetate). The compound was recrystallized from the same solvents (11 mg), mp 178–179 °C (Found: C, 59.84; H, 6.65. $C_{23}H_{30}O_{10}$ requires: C, 59.21; H, 6:50%); IR bands at 1730, 1370, 1240, 1080, 1055, 1035, and 960 cm^{-1}; MS m/e^+ 424, 406, 363, 273, 251, 218, 193 (base peak), 180, 121.

Acetylation of 2. Compound **2** (20 mg) in acetic anhydride (1 mL) and pyridine (1 mL) was kept at room temperature for 20 h. The product was isolated as described for acetylation of neosolaniol. The compound was crystallized from benzene–*n*-hexane (17 mg). The IR, ^1H NMR, and MS spectra of the compound were identical with those of neosolaniol diacetate.

Hydrolysis of 2. Compound **2** (0.2 mg) in 4 N NH_4OH–MeOH (0.4 mL) was kept at room temperature for 17 h. Part of the solution was concentrated, silylated, and subjected to GC–MS. The mass spectrum of the main peak was identical with that of the Me_3Si ether of **7** which was obtained by hydrolysis of neosolaniol.

Hydrolysis of 9. The tetraacetate (10 mg) in 4 mL of aqueous methanolic sodium acetate (10% w/v; pH 9.5) was kept at room temperature with continuous stirring for 1 h. The mixture was resolved on TLC ($CHCl_3$–MeOH, 90:10), yielding a product identical with **2** with respect to TLC, GLC, and mass spectrum.

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., "Microbial Toxins VII", Chapter 7, Academic Press, New York, N.Y., 1971.

Breitmaier, E., Voelter, W., "Monographs in Modern Chemistry", 5, Verlag Chemie, Weinheim/Bergstr., 1974, Chapter 4.
Ellison, R. A., Kotsonis, F. N., *J. Org. Chem.* **41**, 576–578 (1976).
Hsu, Ih-Ch., Smalley, E. B., Strong, F. M., Ribelin, W. E., *Appl. Microbiol.* **24**, 684–690 (1972).
Ishii, K., *Phytochemistry* **14**, 2469 (1975).
Ishii, K., Sakai, K., Ueno, Y., Tsunoda, H., Enomoto, M., *Appl. Microbiol.* **22**, 718 (1971).
Joffe, A. Z., "Microbial Toxins VII", Academic Press, New York, N.Y., 1971, Chapter 5.
Loeffler, W., Mauli, R., Rusch, M. E., Stahelin, H., *Chem. Abstr.* **62**, 5856d (1965).
Loeffler, W., Mauli, R., Rusch, M. E., Stahelin, H., *Chem. Abstr.* **66**, 84744u (1967).
Mirocha, C. J., Christensen, C. M., *Annu. Rev. Phytopathol.* **12**, 303 (1974).
Pathre, S. V., Mirocha, C. J., Christensen, C. M., Behrens, J., *J. Agric. Food Chem.* **24**, 97 (1976).
Sigg, H. P., Mauli, R., Flury, E., Hauser, D., *Helv. Chim. Acta*, **48**, 962 (1965).
Smalley, E. B., Strong, F. M., "Mycotoxins", Elsevier, New York, N.Y., 1974, Chapter 10.

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Inhibition of Formation of Volatile Nitrosamines in Fried Bacon by the Use of Cure-Solubilized α-Tocopherol

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Pork bellies were injected with a conventional cure formulation that would produce target levels of 125 ppm $NaNO_2$ and either 500 ppm sodium ascorbate or α-tocopherol alone or in combination. A mixture of α-tocopherol and Polysorbate 20 (1:0.4 w/w) dispersed in the cure produced a good distribution of α-tocopherol in the adipose tissue which is thought to be the source of nitrosopyrrolidine precursor(s). The volatile nitrosamines in fried bacon were detected by GLC–thermal energy analyzer and confirmed by GLC–high-resolution mass spectrometry. In a comparison of the same sections of each belly pair subjected to different treatments, a combination of α-tocopherol and sodium ascorbate or α-tocopherol alone was found to inhibit nitrosopyrrolidine formation more effectively than did ascorbate alone.

Fried bacon which contains dimethylnitrosamine (DMNA) and nitrosopyrrolidine (NPy) at the ppb (μg/kg) level is still considered a potential source of environmental carcinogens. Since W. Fiddler et al. (1973) demonstrated that sodium ascorbate (NaAsc) and sodium erythorbate (NaEry) markedly diminish formation of DMNA in frankfurters prepared with high levels of nitrite, a number of studies have been made with these reductants in bacon cure. An American Meat Institute–USDA–FDA collaborative study showed that concentrations of NaAsc up to 1000 ppm reduced, but not completely eliminated, NPy in fried bacon prepared with 170 ppm $NaNO_2$ (Greenberg, 1973). The USDA Expert Panel on Nitrosamines (Food Chemical News, 1976) has recommended the use of 125 ppm $NaNO_2$ and 550 ppm NaAsc or NaEry for curing bacon. A study of fried bacon prepared with these concentrations of $NaNO_2$ and NaAsc or NaEry by ten commerical processors showed the effectiveness of this treatment, since only a small number of samples were found to contain very low levels of nitrosamines (NAs) (Food Chemical News, 1977). The mechanism of the inhibitory activity of ascorbate (Asc) or erythorbate (Ery) is thought to be due to their ability to compete with the precursor amine for available N_2O_3 nitrosating species. The limited inhibitory action of these reductants in bacon may be due to the fact that they are more soluble in water than in fat. Nitrosopyrrolidine formation, however, has been shown to be associated with adipose tissue (W. Fiddler et al., 1974; Patterson et al., 1976). Recently, studies have been carried out on the use of lipophilic reductants to inhibit NA formation. Several researchers have determined the effect of a number of compounds on the nitrosation of secondary amines. The compound most commonly used has been ascorbyl palmitate (AscP) on the basis that the palmitoyl ester made Asc more fat soluble. Pensabene et al. (1976) found that this compound and NaAsc inhibited the nitrosation of pyrrolidine in a bacon-like model system containing oil, aqueous buffer, protein, and salts. Mottram and Patterson (1977) in a similar experiment, however, reported an increase in NPy formation with NaAsc and a slight decrease with AscP. The apparent contradiction in results is probably due to the fact that the earlier work was carried out in an open system and the latter in a closed one. The nitric oxide evolved was lost in the open system, thereby making it unavailable for the production of additional nitrosating agent. Despite this, AscP has been found to be more effective than NaAsc in reducing the amount of NPy found in edible bacon, its cooked out fat (Sen et al., 1976a), and vapors during frying (Sen et al., 1976b). In our studies with AscP in bacon we were unable to demonstrate consistent NA inhibition compared to NaAsc (W. Fiddler, 1977). This was possibly due to the slight oil or fat solubility of this compound (Swern, 1949). Compounds more lipophilic than AscP have been investigated in model systems (Gray and Dugan, 1975; Sen et al., 1976a). Coleman (1976) claimed that 5–25 ppm ethoxyquin (6-ethoxy-1,2-di-

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