Occurrence of Scirpentriol and its Seven Acetylated Derivatives in Culture Extracts of Fusarium sambucinum NRRL 13495

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ABSTRACT

Ethyl acetate extracts of culture filtrates from Fusarium sambucinum NRRL 13495 contained scirpentriol, triacetoxyscirpenol, 4,15-diacetoxyscirpenol, 3,4-diacetoxyscirpenol, 3,15-diacetoxyscirpenol, 15-monoacetoxyscirpenol, 4-monoacetoxyscirpenol, and 3-monoacetoxyscirpenol which are the eight possible members of the scirpentriol family of trichothecene mycotoxins. These eight mycotoxins were identified by chemical derivatization, thin layer chromatography, gas chromatography, mass spectroscopy, nuclear magnetic resonance, and comparison to standards. The total concentration of scirpentriol derivatives in the culture filtrate was 426 mg/liter of which 346 mg/L was 4,15-di­acetoxyscirpenol. The occurrence of a complete family of mycotoxins in a single culture implies that outbreaks of toxicity associated with Fusarium mycotoxins in a single culture may be multiple toxicoses, and that the screening of foodstuffs for trichothecenes should be broadened, particularly potato tubers which are subject to infection by F. sambucinum.

Scirpentriol (STO) (3α, 4β, 15-trihydroxy-12,13-epoxytricho­thec-9-ene) and its seven acetylated derivatives comprise a family (Fig. 1) of mycotoxins, some members of which are produced by many species of Fusarium (11). STO has been implicated in cases of mixed toxicoses of animals (15,21) and it has toxicity in the rat dermal assay (3), fibroblast assay (1), and reticulocyte bioassay (12). However, reports of its natural occurrence, along with the occurrence of its derivatives, have been limited (2,13). The best studied of the three possible monoacetylated derivatives in 15-monoacetoxyscirpenol (15-MAS) (15-acetoxy-3α, 4β-dihydroxy-12,13-epoxytrichothec-9-ene). Monoacetoxyscirpenols can also be called monoacetoxyscirpendiols or monoacetylscirpentriols (8). We refer to them as scirpenols because this is the most widely used nomenclature. The correct chemical name based on 12, [3-epoxytrichothec-9-ene is given at first mention of a particular toxin.] 15-MAS causes gastrointestinal hemorrhaging, decreased weight gain, and decreased egg production in chickens and lethality in turkey pouls (16). 3-Monoacetoxyscirpenol (3-MAS) (3α-acetoxy-4β, 15-dihy­droxy-12, 13-epoxytrichothec-9-ene) was found in a rice culture of Fusarium graminearum (1) (F. roseum 'Graminearum'), but its biological activity was not reported (14). 4-Monoacetoxyscirpenol (4-MAS) (4β-acetoxy-3α, 15-dihydroxy-12, 13-epoxytrichothec-9-ene) causes dermal toxicity in rats (8) and has been implicated in mixed toxicoses in ducklings (21), 1,4,5-Diacetoxyscirpenol (4,15-DAS) (4β, 15-diacetoxy-3α-hydroxy-12, 13-epoxytrichothec-9-ene) is the best studied of the STO family of mycotoxins. It elicits toxic responses in swine (27), ducklings (21), chickens (46), horses (26), and rodents (25) and it is suspected of involvement in mixed toxicoses in several species (11). 3,4-Diacetoxyscirpenol (3,4-DAS) (3α, 4β-diacetoxy-15-hydroxy-12,13-epoxytrichothec-9-ene) and 3,15-diacetoxy­scirpenol (3,15-DAS) (3α,15-diacetoxy-4β-hydroxy-12,13­epoxytrichothec-9-ene) as well as the other members of the acetylated STO family were found in a culture of F. equiseti (F. roseum 'Gibbosum'), but details of their isolation, abundance, and biological activities were not reported (14,28). The completely acetylated derivative, 3,4,15-triacetoxyscirpenol (TAS) (3α, 4β,15-triacetoxy-12,13-epoxy­trichothec-9-ene) was implicated in the acute and lethal toxicity of ducklings fed extracts of F. sambucinum (F. sulphureum) (21). TAS, 4,15-DAS, 4-MAS, and 15­MAS were found in maize cultures of F. sambucinum (21). F. sambucinum and its sexual stage, Gibberella pucularis, are important worldwide pathogens of potatoes in which they produce 4,15-DAS and 15-MAS even in apparently healthy tuber tissue adjacent to dry-rotted areas (5,9).

During the preparation of STO and TAS from cultures of F. sambucinum NRRL 13495 (18), we obtained higher yields than quantitative conversion would provide of 4,15­DAS, which was presumed to be the only STO derivative produced by this isolate (17). On investigation with thin
Figure 1. Structures of scirpentriol and its acetylated derivatives.

layer chromatography (TLC) we observed in addition to 4,15-DAS spots which reacted with the 4-(p-nitrobenzyl) pyridine spray for trichothecenes (23) and which had the same color as 4,15-DAS when sprayed with p-anisaldehyde (19). The identities of these additional spots were established, and we now report the occurrence of STO and its seven possible acetylated derivatives in a single culture of F. sambucinum.

MATERIALS AND METHODS

Chemicals

Reagent grade chemicals (Fisher Scientific Co., Raleigh, NC) were used in extraction and chromatography unless otherwise noted. Standard TAS, 15-MAS, and STO were prepared from standard 4,15-DAS (17) according to Sigg et al. (20). The identities of the standards were confirmed by nuclear magnetic resonance (NMR).

Cultural conditions

F. sambucinum NRRL 13495 was maintained on moist autoclaved soil at 5°C. (This fungus was mislabeled as F. roseum NRRL 1181 in the study by Burditt et al. (4). It was recently identified as F. sambucinum by Professor Paul E. Nelson, Fusarium Research Center, Pennsylvania State University, College Park, PA, and accessed by the Northern Regional Research Center, USDA-ARS, Peoria, IL under NRRL 13495). Inoculum for flasks was obtained by sprinkling some of the soil on plates of potato-dextrose agar (Difco Laboratories, Detroit, MI) and incubating at room temperature for 5 d. Blocks of the agar (0.5 cm²) with adherent mycelia served as inoculum for Erlenmeyer flasks (250 ml) containing 40 ml Czapek-Dox broth (Difco) supplemented with 2% peptone (Difco). The flasks were incubated at 29°C as stagnant cultures. After 3 d the liquid portion was decanted and replaced with N-free Czapek-Dox medium. The replacement cultures were incubated an additional 6 d.

Extraction of cultures

Cultures from 25 flasks were filtered through cheesecloth. After adjusting the combined filtrates to 5% with solid NaCl, they were extracted twice with ethyl acetate equal to half their volume. The combined ethyl acetate extracts were evaporated to dryness, dissolved in a small amount of ethyl acetate, transferred to screw cap vials (20 ml), evaporated to dryness under N₂, and stored at room temperature until analyzed.

Preparation of STO and its acetates from TAS

TAS (20 mg) in 5 ml of methanol was added to 25 ml of aqueous 25% sodium acetate in a 100 ml beaker and the pH was adjusted with 0.1 N NaOH to a pH of 7, 8, 9, 10, 11, 12, or 13 and incubated at 60°C in a water bath for 1 h. The hydrolysed mixtures were extracted twice with half-volumes of ethyl acetate which were combined and evaporated to dryness. The residues were redissolved in minimal amounts of ethyl acetate, transferred to vials, evaporated under N₂, and stored until analysis. Doing the hydrolyses under conditions of increasing alkalinity insured that incomplete hydrolysis would be obtained and thus permit determination of the products the acetylated scirpenols were capable of forming.

Isolation of STO and its acetates

Hydrolysis mixtures and culture extracts were screened by spotting on plates of silica gel 60 (Redi-Plates, Fisher Scientific, Raleigh, NC), developing in unlined, unequilibrated tanks with benzene:acetone (3:2, v/v), and visualizing by spraying with 4-(p-nitrobenzyl) pyridine (23), 0.5% p-anisaldehyde in methanol (19), or 25% sulfuric acid in methanol (1) and heating at 110°C for 10 min. STO and its derivatives detected in hydrolysis mixtures and culture extracts were isolated by preparative TLC (0.5 mm layers of silica gel, Uniplates, Analtech, Inc., Newark, DE) using benzene:acetone (3:2, v/v), hexane:ethyl acetate (1:1, v/v), chloroform:methanol (97:3, v/v), or ethyl acetate:toluene (3:1, v/v). The bands of silica gel containing a desired derivative were extracted with ethyl acetate, filtered, and evaporated to dryness. The process was repeated using different developing solvents until a single component was detected by TLC and gas chromatography (GC).

GC

STO and its acetates were quantitated by GC (Model GC-6AM, Shimadzu Scientific Co., Baltimore, MD) using a flame ionization detector (carrier gas was N₂ with a flow rate of 90 ml/min) fueled with hydrogen (70 ml/min) and air (1000 ml/min). Detector and injector were at 300°C. The column (3 mm x 2 m) was packed with 3% OV-17 on 100-120 mesh Gas Chrom Q (Applied Science, State College, PA). The oven was programmed from 150 to 290°C at 5°C/min or 8°C/min with the final temperature held for 10 min. Data were collected on an integrating recorder (Model CR-1A, Shimadzu Scientific Co., Baltimore, MD). Portions of extracts of cultures and hydrolysates were derivatized with 50 µl Tri-Sil TBT (Pierce Co., Rockford, IL) for 0.5 h at 25°C in 2 ml vials fitted with Teflon-lined caps. Volumes of 1-2 µl were injected onto the column. STO and its derivatives were quantitated by the peak area method. A standard curve for each compound was developed by linear regression of the responses for six different concentrations.

Mass spectrometry (MS)

Mass spectra of the isolated compounds were obtained by combination GC/MS (Model HP 5985 mass spectrometer and
data system, Hewlett Packard, Palo Alto, CA) at 230 eV. The GC
column (30m capillary) was coated with DB-1701 (Applied
Science, State College, PA), and the oven was programmed from
180 to 280°C at 3°C/min with the final temperature held for 10
min. Carrier gas was He (1 ml/min) and samples were injected
in the splitless mode. The injector port and detector were at 275
and 200°C, respectively. Positive chemical ionization with methane
was used. The mass range scanned was 100-600 atomic mass
units, and both chromatograms and spectra were reproduced by
the hard copy unit of the computer system.

NMR
Proton NMR spectra of the compounds were determined in
CDCl3, at 300 mHz (Model QE-300, Nicolet, Fremont, CA).
Chloroform was the internal reference at 7.24 ppm. Line broadening
of -0.2 Hz was used.

RESULTS
Alkaline hydrolysis of TAS
The present study was prompted in part by the observation
that acetylation of a crude extract of F. sambuc-inum NRRL 13495 which contained 100 mg 4,15-DAS
yielded 140 mg TAS in contrast to the 111 mg TAS that
quantitative conversion of 4,15-DAS would provide. The
excessive yield implied that part of the TAS arose from
STO derivatives other than 4,15-DAS. TLC of the crude
extract and spraying with p-anisaldehyde revealed a complex
series of overlapping spots of various colors, but four
of the spots had Rf and color identical to TAS, 4,15-DAS,
15-MAS, and STO. Based on the assumption that the other
acetylated derivatives were present, an assumption sup­
ported by the presence of spots reactive with 4-(p-nitroben­
zyl) pyridine spray which is used for the detection of
trichothecenes (23), pure TAS was hydrolyzed under
controlled alkaline conditions to provide hydrolytic prod­
ucts that would serve as standards.

TLC of hydrolysates of TAS revealed the formation
of seven products whose concentration depended on the
pH of the hydrolysis mixture (Fig. 2). Three of the prod­
ucts, (IV, VII, and VIII), migrated with 4,15-DAS, 15-
MAS, and STO. The polarity of the products
and the order of their formation and disappearance during
alkaline hydrolysis implied that products II and III were
diacetylated derivatives and products V and VI were
monoacetylated derivatives of STO. Product V was iden­
tified tentatively as 4-MAS by finding that partial hydroly­
sis of 4,15-DAS yielded STO, 15-MAS and product V.
Consequently, the remaining product (VI) in the region of
monoacetylated derivatives would have to be 3-MAS. Product
II was identified tentatively as 3,4-DAS when partial alkaline
hydrolysis gave TAS and the presumed 4-MAS and 3-
MAS. By the same logic product III should be 3,15-DAS
because it gave rise on partial alkaline hydrolysis to STO,
15-MAS and the presumed 3-MAS. It should be men­
tioned that partial acetylation of STO with acetyl chloride
in pyridine gave the same TLC spots as partial hydrolysis
of TAS.

Figure 2. TLC of alkaline hydrolysates of TAS. Standards of TAS
and 4,15-DAS are on the left and standards of 15-MAS and STO
are on the right. TAS was hydrolyzed at pH 7, 8, 9, 10, 11, 12, or 13. The solvent system was benzene:acetone (3:2) and prod­
ucts I-VIII were detected with p-anisaldehyde.

TLC comparison of TAS and the products of its
hydrolysis with crude culture extracts revealed spots of
matching Rf and color with p-anisaldehyde spray in four
solvent systems (Table 1). GC comparison of TAS and its
hydrolytic products (TMS derivatives) with the compo­
nents in crude culture extracts revealed matching retention
times (Table 2).

Isolation of STO and its derivatives
The compounds were isolated from crude extracts (ethyl
acetate) of culture filtrates by preparative TLC which was
repeated until a single component was detected by TLC
and GC. The diacetates of STO were separated best with
a hexane-ethyl acetate (1:1, v/v) solvent system and the
monoaacetates of STO were separated best with a benzene-
acetone (3:2, v/v) solvent system.

Identification of STO and its derivatives
a) TAS. Chemical ionization MS revealed prominent
peaks at 409 (protonated molecular ion), 408, 349 (base
peak), 307, 289, 259, 247, and 229. NMR resonances were
at δ 0.73 (3H, s, 14-H), 1.69 (3H, s, 16-H), 2.03 (3H, s,
methyl of 15-acetyl), 2.11 (3H, s, methyl of 3-acetyl),
2.08 (3H, s, methyl of 4-acetyl), 2.77 and 3.05 (1H each,
### TABLE 1. TLC behavior of scirpentriol and its acetates

<table>
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<tr>
<th></th>
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<tbody>
<tr>
<td>STO</td>
<td>0.17</td>
<td>0.02</td>
<td>0.04</td>
<td>0.09</td>
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<tr>
<td>15-MAS</td>
<td>0.37</td>
<td>0.05</td>
<td>0.08</td>
<td>0.19</td>
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<tr>
<td>3-MAS</td>
<td>0.52</td>
<td>0.12</td>
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<tr>
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<td>0.50</td>
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<td>3,15-DAS</td>
<td>0.73</td>
<td>0.55</td>
<td>0.43</td>
<td>0.65</td>
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<td>3,4-DAS</td>
<td>0.80</td>
<td>0.65</td>
<td>0.51</td>
<td>0.69</td>
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<tr>
<td>TAS</td>
<td>0.89</td>
<td>0.88</td>
<td>0.71</td>
<td>0.78</td>
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*TLC plates (Redi-Plate silica gel 60) were developed in indicated solvent in an unlined chamber at room temperature. Detection was by spraying with 0.5% p-anisaldehyde and heating at 110°C for 10 min.

### TABLE 2. GC retention times of TMS derivatives of scirpentriol and its acetates

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
</tr>
</thead>
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<td></td>
<td>OV-17 Column</td>
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<tr>
<td>STO</td>
<td>14.08</td>
</tr>
<tr>
<td>15-MAS</td>
<td>13.75</td>
</tr>
<tr>
<td>3-MAS</td>
<td>13.95</td>
</tr>
<tr>
<td>4-MAS</td>
<td>15.08</td>
</tr>
<tr>
<td>4,15-DAS</td>
<td>15.68</td>
</tr>
<tr>
<td>3,15-DAS</td>
<td>15.38</td>
</tr>
<tr>
<td>3,4-DAS</td>
<td>17.42</td>
</tr>
<tr>
<td>TAS</td>
<td>14.08</td>
</tr>
</tbody>
</table>

### Experimental Procedures

When subjected to MS, the TMS derivative gave prominent peaks at 439 (protonated molecular ion), 438 (molecular ion), 423, 379 (base peak), 319, 301, 289, and 229. NMR resonances were at 6 0.75 (3H, s, 14-H), 1.66 (3H, s, 16-H), 1.99 (3H, s, methyl of 15-acetyl), 2.08 (3H, s, methyl of 4-acetyl), 2.73 and 3.01 (1H each, AB, J=4.0, 13-H), 3.63 (1H, d, J=4.9, 2-H), 3.91 and 4.11 (1H each, AB, J=12.4, 15-H), 4.05 (1H, d, J=5.4, 11-H), 4.12 (1H, dd, J=2.9 and 4.9, 3-H), 5.10 (1H, d, J=2.9, 4-H), and 5.48 (1H, d, J=5.4, 10-H). These physical properties closely match prior reports of 4,15-DAS (16,20).

**Prominent peaks at** 439 (protonated molecular ion), 438 (molecular ion), 423, 379 (base peak), 319, 301, 289, and 229 were displayed by its TMS derivative. NMR revealed resonances at 6 0.83 (3H, s, 14-H), 1.71 (3H, s, 16-H), 2.14 (3H, s, methyl of 4-acetyl), 2.77 and 3.05 (1H each, AB, J=4.0, 13-H), 3.61 and 3.78 (1 each, dd, J=3.71=12.0, J=3.78=5.5, 15-H), 3.66 (1H, d, J=4.6, 2-H), 4.18 (1H, d, J=5.5, 11-H), 4.25 (1H, dd, J=3.1 and 4.6, 3-H), 5.50 (1H, d, J=3.1, 4-H), and 5.56 (1H, d, J=5.5, 10-H). NMR characteristics match closely those reported by Ishii et al. (8).

### MS Properties

MS of the TMS derivative showed strong ion peaks at 4.69 (protonated molecular ion), 468 (molecular ion), 453 (base peak), 379, 319, 301, 295, 289, 277, 257, and 229. NMR resonances were at 6 0.92 (3H, s, 14-H), 1.71 (3H, s, 16-H), 2.20 (3H, s, methyl of 3-acetyl), 2.79 and 3.05 (1H each, AB, J=4.0, 13-H), 3.53 and 3.74 (1H each, AB, J=11.8, 15-H), 3.74 (1H, d, J=3.5, 11-H), 3.77 (1H, d, J=4.9, 2-H), 4.49 (1H, d, J=3.0, 4-H), 4.83 (1H, dd, J=3.0 and 4.9, 3-H), and 5.47 (1H, d, J=5.3, 10-H). These properties have not been reported for 3-MAS before.

### Properties of 3-MAS

MS of the TMS derivative showed strong ion peaks at 4.69 (protonated molecular ion), 468 (molecular ion), 453, 409, 379, 319, 303, 265 (base peak), and 217. NMR resonances were at 6 0.78 (3H, s, 14-H), 1.69 (3H, s, 16-H), 2.03 (3H, s, methyl of 15-acetyl), 2.72 and 3.01 (1H each, AB, J=3.9, 13-H), 3.61 (1H, d, J=4.7, 2-H), 3.92 (1H, d, J=5.4, 11-H), 4.19 and 4.83 (1H each, AB, J=12.1, 15-H), 4.20 (1H, dd, J=2.8 and 4.7, 3-H), 4.26 (1H, dd, J=2.3 and 2.8, 4-H), and 5.47 (1H, d, J=5.4, 10-H). These physical properties are similar to those reported by Pathre et al. (16).

### Properties of 15-MAS

MS of the TMS derivative showed strong ion peaks at 4.69 (protonated molecular ion), 468 (molecular ion), 453, 409, 379, 319, 303, 265 (base peak), and 217. NMR resonances were at 6 0.89 (3H, s, 14-H), 1.71 (3H, s, 16-H), 2.74 and 3.01 (1H each, AB, J=3.9, 13-H), 3.54 and 3.77 (1H each, AB, J=4.8 and 11.8, 15-H), 3.60 (1H, d, J=4.6, 2-H), 4.09 (1H, d, J=5.4, 11-H), 4.20 (1H, dd, J=2.8 and 4.6, 3-H), 4.45 (1H, dd, J=2.4 and 2.8, 4-H) and 5.49 (1H, d, J=5.4, 10-H). These physical properties closely match prior reports (16,20).
Quantitation of STO and derivatives

GC analysis of extracts after forming the TMS derivatives revealed concentrations of STO and its seven acetylated derivatives in the culture filtrates ranging from barely detectable amounts of TAS to 346 mg 4,15-DAS/L (Table 3). The total concentration of STO and its derivatives was 426 mg/L of culture filtrate.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg/L)</th>
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<tbody>
<tr>
<td>STO</td>
<td>12.9 ± 1.8a</td>
</tr>
<tr>
<td>15-MAS</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>3-MAS</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>4-MAS</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>4,15-DAS</td>
<td>346 ± 14</td>
</tr>
<tr>
<td>3,15-DAS</td>
<td>47.9 ± 2.8</td>
</tr>
<tr>
<td>3,4-DAS</td>
<td>7.2 ± 1.1</td>
</tr>
<tr>
<td>TAS</td>
<td>0.24 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± standard errors of three replicates.

DISCUSSION

The production of multiple mycotoxins by a single culture appears to be the rule rather than the exception (24), but production of entire families of mycotoxins has fewer precedents. The family of aflatoxins B1, B2, G1, and G2 occur together commonly (22), as do ochratoxins A, B, and C which comprise another family of biosynthetically related mycotoxins (5). The production of all members of the scirpentriol family by a single culture of F. equiseti (F. roseum ‘Gibbosum’) has been reported before (28) but without supporting data. Mirocha et al. (14) documented that F. graminearum (F. roseum ‘Graminearum’) produced STO, TAS, 4,15-DAS, and the three MAS isomers, while F. equiseti produced the three isomers of DAS. The simultaneous occurrence of closely related members of a family of mycotoxins such as the acetylated STO derivatives implies possible precursor-product relationships; however, the present investigation was not a study of kinetics and neither an acetylation nor deacetylation pathway can be supported. The simultaneous occurrence of the eight members also offers the prospect of isolating useful quantities of the rarer members.

The current results confirm prior reports that F. sambucinum produces notable quantities of 4,15-DAS and detectable amounts of 15-MAS, 4-MAS, STO, and TAS (20,21). The relative abundance of the MAS derivatives and of the DAS derivatives differed from those reported in F. graminearum and F. equiseti (14). For the MAS series in F. graminearum (14), the order of abundance was 15-MAS > 4-MAS > 3-MAS, while the order in F. sambucinum (Table 3) was 15-MAS > 3-MAS > 4-MAS. For the DAS series in F. equiseti, the order of abundance was 4,15-DAS > 3,4-DAS > 3,15-DAS, while the order in F. sambucinum was 4,15-DAS > 3,15-DAS > 3,4-DAS. The difference in relative abundance may be related to species, or they may reflect differences in conditions of incubation which were not reported for the earlier study (14).

The methods used here to separate and detect acetylated derivatives of STO in culture filtrates of F. sambucinum NRRL 13495 appear simple enough for application to screening other cultures for production of STO derivatives. An ethyl acetate extract could be subjected to TLC (Table 1) or GC (Table 2) and compared to eight standards prepared by partial hydrolysis or acetylation of STO or one of the acetylated derivatives (Fig. 2). Simple derivatization of STO or its presumptive acetates in an extract to another acetylated derivative would offer a higher level of proof. If necessary, NMR or GC/MS confirmation could be done. In regard to GC/MS of STO and its acetates, we found that TMS derivatives of 3-MAS and 4-MAS and of 3,4-DAS and 4,15-DAS yielded spectra easily distinguished from each other. In contrast, Mirocha et al. (14) found that trifluoroacetyl derivatives of these compounds had similar mass spectra and they relied on chromatography rather than mass spectra to resolve the isomers.

The occurrence of STO and its seven acetylated derivatives in cultures of F. sambucinum emphasizes a new the undesirability of working with unknown mixtures of toxins if toxic responses are to be assigned to a single compound. For example, crude culture filtrates of F. sambucinum NRRL 13495 containing 4,15-DAS, the only mycotoxin detected at the time, was used to demonstrate that feed refusal syndrome in chickens could be caused by fungi (4). Obviously, feed refusal caused by this culture should be investigated further. The simultaneous occurrence of a complete family of mycotoxins also emphasizes the need to investigate the toxicity of mixtures of mycotoxins, since interactions between mycotoxins have been well demonstrated as a phenomenon (10). The current findings also imply that suspect feeds and foods could be examined profitably for STO and its acetates. Because potato tubers infected with F. sambucinum have been associated with esophageal cancer (21), they would appear to be particularly suitable for increased surveillance.

REFERENCES


