# Mycotoxins Produced by *Fusarium acuminatum*. Isolation and Characterization of Acuminatin: A New Trichothecene

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A new trichothecene—acuminatin—has been isolated from a culture of *Fusarium acuminatum* Ell. & Ev. (strain ITEM 484) on corn and identified as 3$\alpha$,4$\beta$-dihydroxy-8$\alpha$,15-diacetoxy-12,13-epoxytrichothec-9-ene. The same culture also produced 8-acetoxyneosolaniol (the major metabolite) and other poly- and monoacetylated trichothecenes, namely neosolaniol, 4,8-diacetoxy T-2 tetraol, tetraacetoxy T-2 tetraol, and 4-, 8-, or 15-acetoxy T-2 tetraol. Culture extracts of eight strains of *F. acuminatum* isolated in Italy from soil and corn stalk or ear contained considerable amounts of trichothecenes, were highly toxic to *Artemia salina* larvae, and inhibited the growth of tomato seedlings. The trichothecene production pattern of these isolates (mainly related to 8-acetoxyneosolaniol) was different from that of other isolates of the same species reported in the literature, producing mainly T-2 toxin and its derivatives.

*Fusarium acuminatum* Ell. & Ev. is a fungus occurring worldwide as a soil saprophyte and as a secondary invader associated with root, foot, and stem rot of a great variety of plants in temperate and tropical areas (Marasas et al., 1984). During the screening of various *Fusarium* species for toxicity (vs *Artemia salina* and tomato seedlings) and mycotoxin production, we found *F. acuminatum* to be one of the most toxic in addition to *Fusarium sporotrichioides* (Logrieco et al., 1989). Although none of the major trichothecenes looked for (including T-2 toxin, HT-2 toxin, diacetoxyscirpenol, fusarenone, deoxynivalenol, and 3-acetyldeoxynivalenol) was detected in the culture extracts of isolates of *F. acuminatum*, the thin-layer chromatographic (TLC) analysis of such extracts revealed the presence of a number of compounds positive to the characteristic reaction of trichothecenes with (*p*-nitrobenzyl)pyridine (Takitani et al., 1979). In this paper the isolation and identification of such compounds from a culture of *F. acuminatum* isolated from corn stalk is described, with particular emphasis on the characterization of the new trichothecene acuminatin. The production of trichothecenes by other strains of the same species isolated from corn field in Italy and their potential toxicity are also reported.

## EXPERIMENTAL SECTION

**Fungal Source and Culturing.** Eight strains of *F. acuminatum* Ell. & Ev. were isolated from corn stalks (two strains), corn ear (one strain), and soil (five strains) in Italy and identified in accordance with the taxonomic system of Nelson et al. (1983). These isolates have been recently classified at the Research Institute for Nutritional Diseases, Tygerberg, South Africa (Marasas, W. H. O., personal communication) as *Fusarium compactum* (Wollenw.) Gordon, a species not well-defined in the Nelson et al. (1983) system. At present, we maintain the classification *F. acuminatum*, which is also in agreement with the taxonomic system of Booth (1971). The isolates were grown on 100 g of corn kernels brought to about 45% moisture in 500-mL Erlenmeyer flasks and then autoclaved at 120 °C for 20 min. The medium was inoculated with fungal cultures from potato–dextrose–agar and incubated at 25–27 °C for 4 weeks. Then, the bulk cultures were dried at 60 °C for 48 h and finely ground. Trichothecenes were extracted according to a procedure previously reported (Bottalico et al., 1983) and detected by thin-layer chromatography (TLC) as described below.

**Table I. HPTLC $R_f$ Values of Polyacetylated Trichothecenes Produced by *F. acuminatum***

| no. | trichothecene | A[a] | B | C |
|---|---|---|---|---|
| 5 | tetraacetoxy T-2 tetraol | 0.83 | 0.93 | 0.45 |
| 1 | 8-acetoxyneosolaniol | 0.6 | 0.85 | 0.28 |
| 4 | 4,8-diacetyoxy T-2 tetraol | 0.51 | 0.73 | 0.22 |
| 3 | neosolaniol | 0.4 | 0.62 | 0.15 |
| 2 | acuminatin | 0.24 | 0.48 | 0.12 |

[a] Key: A = benzene–acetone (12:7); B = chloroform–methanol (9:1); C = toluene–ethyl acetate–formic acid (6:3:1)

**Production and Isolation of Trichothecenes from *F. acuminatum* Strain ITEM 484.** Strain ITEM 484 was grown on 2 kg of corn kernels distributed in 20 flasks. A first extraction was performed with 5 L of MeOH–1% aqueous NaCl (55:45) in a rotary shaker at 250 rpm for 18 h. After filtration, the residue was extracted in a Waring Blendor with 3 L of MeOH–NaCl solution. The filtrates were combined and concentrated to about 3 L in a rotary evaporator at 60 °C. The residue was defatted twice with *n*-hexane and then extracted four times with 1.2 L of $CH_2Cl_2$. The combined $CH_2Cl_2$ phases were dried over anhydrous $Na_2SO_4$ and concentrated to dryness. The residue was reconstituted with 20 mL of MeOH and chromatographed on a 50-cm column (3-cm i.d.) packed with silica gel 60 (Merck, Darmstadt, FRD). The column was eluted sequentially with 2 L of $CH_2Cl_2$, 1.5 L of $CH_2Cl_2$–MeOH (95:5), 1.5 L of $CH_2Cl_2$–MeOH (90:10), 1.5 L of $CH_2Cl_2$–MeOH (80:20), and 1.5 L of MeOH. Eighteen fractions (F1–F18) of about 450 mL each were collected and tested by TLC for trichothecenes. The compounds of major interest, positive to trichothecene reaction, accumulated in F6 and F7. In particular, fraction F6 contained compounds **1**–**5** (later identified as 8-acetoxyneosolaniol, acuminatin, neosolaniol, 4,8-diacetoxy T-2 tetraol, and tetraacetoxy T-2 tetraol, respectively), and fraction F7 contained mostly compound **2** (see Figure 1 for chemical structures and Table I for $R_f$ values). These compounds were purified from F6 and F7 by preparative high-performance liquid chromatography (HPLC) performed on a 250 × 25 mm RP-18 column with MeOH–$H_2O$ (40:60) as eluent at a flow rate of 15 mL/min (apparatus: Perkin-Elmer Series 3B liquid chromatograph). Eluted subfractions (f1–f24) were collected every 54 s and tested for trichothecenes by TLC. Subfractions f18 and f19 obtained from F7 contained only compound **2**, which was also eluted together with another component (higher $R_f$ value in 12:7 benzene–acetone) in f13–f17. The following resulted from HPLC purification of F6: compound **1** in f4–f10; compounds **1** and **5** in f11 and f12; compounds **1**, **3**, and **5** in f13 and f14; compound **5** in f15 and f16; compounds **2** and **4** in f17–f19. Subfractions

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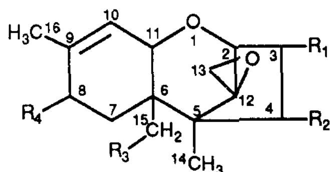


| no. | compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|
| 1 | 8-acetoxyneosolaniol | OH | OAc | OAc | OAc |
| 2 | acuminatin | OH | OH | OAc | OAc |
| 3 | neosolaniol | OH | OAc | OAc | OH |
| 4 | 4,8-diacetoxy T-2 tetraol | OH | OAc | OH | OAc |
| 5 | tetraacetoxy T-2 tetraol | OAc | OAc | OAc | OAc |
| 6 | 4-acetoxy T-2 tetraol | OH | OAc | OH | OH |
| 7 | 8-acetoxy T-2 tetraol | OH | OH | OH | OAc |
| 8 | 15-acetoxy T-2 tetraol | OH | OH | OAc | OH |

**Figure** 1. Chemical structure of trichothecenes produced by *F. acuminatum* ITEM 484.

containing the same components were put together and lyophilized, after methanol evaporation under nitrogen stream. Pure compounds 1, 2, and 5 were obtained at levels of 70, 57, and 5 mg, respectively. Fractions containing a mixture of trichothecenes were reconstituted in MeOH and purified by preparative TLC to obtain pure 3 (18 mg) and 4 (7 mg).

**Thin-Layer Chromatography.** High-performance TLC (HPTLC) silica gel precoated plates (10 × 10 cm; Merck) were used for semiquantitative analysis of trichothecene produced by the various *Fusarium* isolates. The following solvent systems were used: A, benzene–acetone (12:7); B, chloroform–methanol (9:1); C, toluene–ethyl acetate–formic acid (6:3:1). Trichothecenes were detected either by fluorescence under UV light after spraying with $H_2SO_4$ and heating or by the blue color developed after reaction with (*p*-nitrobenzyl)pyridine (Takitani et al., 1979). Silica gel precoated (0.5 mm) preparative TLC plates (20 × 20 cm; Merck) were eluted in solvent A for purification of HPLC fractions.

**Gas Chromatography.** An aliquot of culture extract of strain ITEM 484 was concentrated to dryness and reacted with 50 μL of TFAA (trifluoroacetic anhydride; Pierce Chemical Co.) at 60 °C for 30 min. After reagent excess was eliminated under a nitrogen stream, the residue was reconstituted with 50 μL of toluene, and 1 μL was injected in the gas chromatograph. Trichothecene analysis was performed by comparison with TFA derivatives of reference standards obtained with the same procedure. The GC apparatus was a Hewlett-Packard 5840A equipped with a flame ionization detector and a DB5 capillary column (30 m × 0.25 mm) with covalently bonded liquid phase. GC conditions: injector temperature, 275 °C; detector temperature, 300 °C; temperature programming, 150–280 °C at 10 °C/min followed by 5 min at 280 °C.

**Mass Spectrometry.** Methane (source pressure 0.6 Torr) positive chemical ionization (CI) mass spectra were performed on underivatized trichothecenes by direct-probe inlet on a VG 7070 EQ MS/MS apparatus, at 200 eV. Electron impact (EI) spectra were also recorded on the same instrument at 70 eV. GC/MS analysis of TFA derivatives was performed on a Hewlett-Packard Model 5987B with the same GC conditions as above and methane CI.

**Nuclear Magnetic Resonance.** Proton NMR spectra were carried out on a Bruker NMR apparatus at 200 MHz, using $CDCl_3$ as solvent and TMS as calibrant. $^{13}C$ NMR spectra were obtained at 50.3 MHz on a Varian XL-200 NMR spectrometer.

**Chemical Reactions.** *Hydrolysis of 1, 2, and 4.* Compounds 1, 2, and 4 (about 0.3 mg each) were separately hydrolyzed with 4 N $NH_4OH$–MeOH (0.5 mL) at room temperature for 20 h. T-2 tetraol was obtained as the only hydrolysis product and confirmed by TLC in solvents A–C.

*Acetylation of 2.* A mixture of compound 2 (2 mg), acetic anhydride (0.5 mL), and pyridine (0.5 mL) was kept at room temperature for 24 h, then 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 reaction product was identical with compound 5 as confirmed by TLC and MS analysis.

*Acetylation of 1.* Compound 5 was also obtained from the acetylation of compound 1 performed as described for 2.

**Table II. Diagnostic Ions Derived by Positive Chemical Ionization (Methane) Mass Spectrometry of Some Trichothecene TFA Derivatives**

| | $MH^{+a}$ | $-HR_8$[b] | $-HR_{15}$ | $-HR_4$ | other ions |
|---|---|---|---|---|---|
| 1[c] | 521 (8) | 461 (10) | 401 (100) | 341 (10) | |
| 2 | 575 (16) | 515 (8) | 455 (100) | 341 (8) | 395, 311 |
| 3 | 575 (4) | 461 (8) | 401 (100) | 341 (10) | 347, 287 |
| 4 | 575 (20) | 515 (100) | 401 (45) | 341 (20) | 455 |
| T-2 toxin | 563 (4) | 461 (6) | 401 (100) | 341 (10) | |
| HT-2 toxin | 617 (15) | 515 (14) | 455 (100) | 341 (12) | 395, 311 |

[a] Protonated molecular ion. Relative intensities are given in parentheses. [b] $HR_8$, $HR_{15}$, and $HR_4$ represent the acids lost by fragmentation at C-8, C-15, and C-4, respectively, which may be acetic (60 amu), isovaleric (102 amu), or trifluoroacetic (114 amu) acid. Ions reported in each raw are relevant to sequential losses of HRs. [c] Key: 1 = 8-acetoxyneosolaniol; 2 = 8,15-diacetoxy T-2 tetraol (acuminatin); 3 = 4,15-diacetoxy T-2 tetraol (neosolaniol); 4 = 4,5-diacetoxy T-2 tetraol (NT-1).

**Bioassays.** *Brine Shrimp. Artemia salina* eggs were purchased from Euraquarium (Bologna, Italy) and left to hatch for 36 h in one sector of a special four-sector Petri dish filled with marine water (3.3% sea salt solution). The sectors were communicating through horizontal slits (1 mm × 3 cm) practised in the middle of the partitions to allow brine shrimps to move out of the hatching sector after disclosure of larvae. Bioassays were performed on 24-well cell culture plates (Corning, NY) containing about 30–40 shrimp in 500 μL of marine water and 1% methanolic extract of fungal culture. The equivalent amounts of original culture tested (four replicates) were 50 and 5 mg/500 μL of marine water, respectively. The number of dead shrimp was recorded by reversed microscopy after incubation at 27 °C for 36 h. The total number of shrimps per well was measured after the remaining shrimp were killed by freezing at −20 °C for 12 h.

*Tomato Seedlings.* Tomato seeds (var. Marmande) were sterilized with 4% NaClO for 10 min and left to germinate on water-impregnated filter paper for 3 days at room temperature. Ten germinating seedlings were selected per test and put in a Petri dish (φ 5 cm) containing the same size filter paper. A 2-mL portion of water containing 1% MeOH (controls) or methanolic extracts equivalent to 200 mg of original culture (tests) were added, and root and shoot elongation measurements were made after 4-day incubation at room temperature. Growth inhibition was expressed as percent vs control length. Three replicates per test were performed.

## RESULTS

**Characterization of Acuminatin.** The new trichothecene from *F. acuminatum* (compound 2) was first detected by positive reaction with (*p*-nitrobenzyl)pyridine or $H_2SO_4$ on the TLC plates. Its $R_f$ value in various solvent systems is reported in Table I, together with those relevant to other trichothecenes also found in the culture extracts of *F. acuminatum*. The mass spectrum (EI mode) showed a weak parent peak at $m/z$ 382 and main fragment ions at $m/z$ 323 (− 1 acetate) and 263 (− 2 acetate). Protonated molecular ion ($MH^+$) was clearly evident in CI mode at $m/z$ 383 together with a series of diagnostic fragment ions formed by loss of AcOH and/or $CH_2O$ ($m/z$ 323, 263, 233, 203). Reaction with TFAA gave rise to a compound with MW (molecular weight) 574 ($MH^+$ = 575 observed by CI MS), indicating the presence of two reacted hydroxy groups in the molecule. The reaction products of the complete hydrolysis and acetylation of compound 2 were T-2 tetraol and tetraacetoxy T-2 tetraol, respectively. The above results clearly indicated that the new compound was an isomer of neosolaniol (compound 3) and NT-1 (compound 4) (MW = 382) also having two hydroxy and two acetoxy groups. A first indication on the position of the acetates was obtained from the CI MS fragmentation of the TFA derivative, showing the loss of two molecules of AcOH from C-8 and C-15 respectively, and one molecule of TFA from C-4. In fact, by assuming that the frag-

Table III. Chemical Shifts (δ) of Protons[a] in Major Trichothecenes Produced by *F. acuminatum*

| | 1[b] | 2 | 3 | 4 |
|---|---|---|---|---|
| H-2 | 3.69 (5.0) | 3.62 (5.0) | 3.69 | 3.66 |
| H-3 | 4.17 (5.0, 3.0) | 4.25 (5.0, 3.0) | 4.2 | 4.24 |
| H-4 | 5.25 (3.0) | 4.34 | 5.27 | 5.40 |
| H-7a | 2.15 (15) | 2.15 (15) | 2.18 | 1.96 |
| H-7b | 2.37 (15, 5.5) | 2.28 (15, 5.5) | 2.18 | 2.34 |
| H-8 | 5.23 (5.0) | 5.24 (5.0) | 4.2 | 5.36 |
| H-10 | 5.80 (6.0) | 5.76 (5.5) | 5.68 | 5.82 |
| H-11 | 4.29 (6.0) | 4.07 (5.5) | 4.2 | 4.27 |
| H-13a | 2.79 (4.0) | 2.77 (4.0) | 2.79 | 2.80 |
| H-13b | 3.06 (4.0) | 3.03 (4.0) | 3.04 | 3.04 |
| H-14 | 0.81 | 0.78 | 0.86 | 0.84 |
| H-15a | 4.06 (12.5) | 3.97 (12.5) | 4.1–4.3 | 3.60 |
| H-15b | 4.25 (12.5) | 4.32 (12.5) | 4.1–4.3 | 3.91 |
| H-16 | 1.75 | 1.74 | 1.87 | 1.75 |
| OAc | 2.03; 2.03; 2.14 | 2.02; 2.04 | 2.04; 2.15 | 2.09; 2.14 |

[a] In $CDCl_3$ solution. In ppm downfield from internal $Me_4Si$. Proton–proton coupling constants in hertz given in parentheses. [b] Key: 1 = 3-hydroxy-4,8,15-triacetoxy-12,13-epoxytrichothec-9-ene (8-acetoxyneosolaniol); 2 = 3,4-dihydroxy-8,15-diacetoxy-12,13-epoxytrichothec-9-ene (acuminatin, 8,15-diacetoxy T-2 tetraol); 3 = 3,8-dihydroxy-4,15-diacetoxy-12,13-epoxytrichothec-9-ene (neosolaniol); 4 = 3,15-dihydroxy-4,8-diacetoxy-4,8-diacetoxy-12,13-epoxytrichothec-9-ene (NT-1, 4,8-diacetoxy T-2 tetraol).

mentation of trichothecene TFA derivatives in CI mode occurs by sequential loss of substituents at C-8, C-15, and C-4 [as shown in Table II representing the major fragment ions for a number of trichothecenes closely related to compound **2**; data relevant to T-2 toxin and HT-2 toxin were obtained from Mirocha et al. (1986)], the following can be stated. The fragment ion at $m/z$ 401 (observed in the case of T-2 toxin and compounds **1**, **3**, and **4**; Table II) is indicative of an acetate at C-4 retained in such fragment, while $m/z$ 455 (base peak for HT-2 toxin and compound **2**) indicates the presence of a TFA-reacted hydroxy group at C-4; the loss of the substituent at C-4 (AcOH or TFA) produces $m/z$ 341 in both cases. Mass spectra of HT-2 toxin and compound **2** are quite similar, the only difference being the parent ion ($m/z$ 617 and 575, respectively); the loss of substituent at C-8 (isovaleric and acetic acid for HT-2 toxin and compound **2**, respectively) gives rise to fragment 515 and related daughter ions identical for both compounds. The ultimate proof of the position of the acetate and hydroxy groups in the molecule of the new compound was provided by the proton NMR analysis and its comparison with compounds **1**, **3**, and **4** [Table III; data relevant to compounds **3** and **4** obtained from Cole and Cox (1981)]. The H-3 chemical shift of compound **2** (δ 4.25) was similar to that of compounds **1**, **3**, and **4** indicating a bonded hydroxy group, while the H-4 chemical shift (δ 4.34) was different from the other three compounds, showing a downfield shift of about 1 ppm due to the acetylation of the bonded hydroxy group. Chemical shifts of H-8, H-15a, and H-15b (δ 5.24, 3.97, and 4.32, respectively) were consistent with the presence of a bonded acetate group as in the case of compound **1**. The following $^{13}C$ chemical shift (ppm) assignments were made for compound **2**: C-2 (78.64), C-3 (80.65), C-4 (81.66), C-5 (46.82), C-6 (42.43), C-7 (27.10), C-8 (68.53), C-9 (136.25), C-10 (123.74), C-11 (67.30), C-12 (64.56), C-13 (49.01), C-14 (7.02), C-15 (64.30), C-16 (20.30), $CH_3CO$ (21.07), $CH_3CO$ (170.38, 170.59). From the above data, the new trichothecene (compound **2**) has been shown to be 3α,4β-dihydroxy-8α,15-diacetoxy-12,13-epoxytrichothec-9-ene, and we assigned it the trivial name acuminatin (or 8,15-diacetoxy T-2 tetraol).

**Identification of Other Trichothecenes Produced by *F. acuminatum* ITEM 484.** Compounds **1** and **3–5** were also isolated from the bulk culture of *F. acuminatum* ITEM 484 (see Table I for TLC $R_f$ values) and identified by MS. In particular $MH^+$ ions were observed (by direct-probe CI MS) at $m/z$ 425, 383, 383, and 467, for compounds **1** and **3–5**, respectively, corresponding to 8-acetoxyneosolaniol, neosolaniol, 4,8-diacetoxy T-2 tetraol, and tetraacetoxy T-2 tetraol (see Figure 1 for structures). The TFA derivatives of compounds **1**, **3**, and **4** showed $MH^+$ at $m/z$ 521, 575, and 575, respectively. Hydrolysis of compounds **1** and **4** produced T-2 tetraol. Acetylation of compound **1** gave rise to compound **5**. All the above *F. acuminatum* metabolites were positive to typical trichothecene reactions with (*p*-nitrobenzyl)pyridine or $H_2SO_4$. Also, monoacetylated trichothecenes, namely 4-, 8-, and 15-acetoxy T-2 tetraol (compounds **6–8**, respectively; see Figure 1 for structures), were produced by ITEM 484 together with the above polyacetylated ones. A list of trichothecenes detected by GC in a culture extract of *F. acuminatum* ITEM 484 is reported in Table IV. No T-2 toxin, HT-2 toxin, DAS, deoxynivalenol, 3-acetyldeoxynivalenol, and fusarenone were detected.

Table IV. TFA Derivative Retention Times and Concentrations of Trichothecenes Found in a Culture of *F. acuminatum* ITEM 484 by Gas Chromatography[a]

| no. | compound | ret time[b] | concn[c] |
|---|---|---|---|
| 8 | 15-acetoxy T-2 tetraol | 9.37 | 6.4 |
| 7 | 8-acetoxy T-2 tetraol | 9.56 | 12.4 |
| 6 | 4-acetoxy T-2 tetraol | 9.92 | 2.4 |
| 2 | acuminatin | 10.51 | 100.8 |
| 3 | neosolaniol | 10.72 | 6.8 |
| 4 | 4,8-diacetoxy T-2 tetraol | 10.85 | 11.2 |
| 1 | 8-acetoxyneosolaniol | 11.86 | 176.0 |
| 5 | tetraacetoxy T-2 tetraol | 13.38 | 3.6 |

[a] Conditions: DB5 capillary column (30 m × 0.25 mm); injector temperature, 275 °C; detector temperature, 300 °C; temperature programming, 150–280 °C at 10 °C/min and then 5 min at 280 °C. [b] Minutes. [c] Milligrams per kilogram.

Table V. Growth Inhibition of Tomato Shoot and Root by Culture Extracts of Some *F. acuminatum* Strains[a]

| strain | shoot growth inhibn, % | root growth inhibn, % |
|---|---|---|
| ITEM 484 | 47 | 90 |
| ITEM 488 | 52 | 88 |
| ITEM 490 | 42 | 81 |
| ITEM 491 | 26 | 81 |
| ITEM 492 | 34 | 81 |
| ITEM 493 | 36 | 81 |
| ITEM 494 | 36 | 81 |
| ITEM 495 | 36 | 82 |
| mean (SD) | 39.8 (6.1) | 83.1 (3.4) |

[a] 1% MeOH solutions containing 100 mg equivalent of culture/mL, tested on 10 tomato germinating seeds. Measurements (three replicates) were made after 4-day incubation. All the extracts were toxic to brine shrimp larvae (100% mortality) at levels of 5 mg of culture/0.5 mL.

**Toxicity and Trichothecene Production by *F. acuminatum* Isolates from Corn Field.** The culture extracts of various strains of *F. acuminatum* isolated in Italy from corn field (soil, corn stalk or ear) were shown to be quite toxic to *A. salina* and inhibited the growth of tomato seedlings (Table V). All the tested isolates produced on corn kernels considerable amounts of the above-mentioned trichothecenes (Table VI), which were the major ones responsible for the strong biological activity. Compounds **1** and **2** were the main metabolites produced on corn at levels higher than 100 ppm. The monoacetylated compounds **6–8** were not looked for in the culture extracts of *F. acuminatum* strains other than ITEM 484. Other important trichothecenes, including T-2 toxin, HT-2 toxin,

**Table VI. Production of Polyacetylated Trichothecenes (1–5) by Some Strains of *F. acuminatum* Isolated In Italy**[a]

| strain | source | 1[b] | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| ITEM 484 | corn stalk | 175[c] | 100 | 10 | 10 | 10 |
| ITEM 488 | corn stalk | 200 | 100 | 10 | 10 | 10 |
| ITEM 490 | corn kernel | 40 | 100 | 10 | 10 | 10 |
| ITEM 491 | soil | 150 | 100 | 10 | 10 | 10 |
| ITEM 492 | soil | 100 | 125 | 40 | 10 | 10 |
| ITEM 493 | soil | 125 | 50 | 10 | 10 | 10 |
| ITEM 494 | soil | 175 | 150 | 10 | 10 | 10 |
| ITEM 495 | soil | 200 | 100 | 10 | 10 | 40 |

[a] Cultured on autoclaved corn at 25–27 °C for 4 weeks. [b] Key: 1 = 8-acetoxyneosolaniol; 2 = acuminatin; 3 = neosolaniol; 4 = 4,8-diacetoxy T-2 tetraol; 5 = tetraacetoxy T-2 tetraol. [c] Milligrams per kilogram. Detected by TLC.

diacetoxyscirpenol, and 8-ketotrichothecenes, were not present at levels higher than the TLC detection limit.

## DISCUSSION

This is the first report on the production of compounds **2** (acuminatin or 8,15-diacetoxy T-2 tetraol) and **5** (tetraacetoxy T-2 tetraol) by any fungal species and compounds 1 (8-acetoxyneosolaniol) and **4** (NT-1 or 4,8-diacetoxy T-2 tetraol) by *F. acuminatum*.

The few data available in the literature on toxigenicity of *F. acuminatum* refer to a very limited number of isolates of this species, in which are also included, according to revised nomenclature (Marasas et al., 1984), the strains of *Fusarium heterosporum* F-77-1A (Cole et al., 1981), and *Fusarium sulphureum* HPB 110178-19 (Harwig et al., 1979). Such data report the production of T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol, 3′-hydroxy T-2 toxin, 3′-hydroxy HT-2 toxin, 3′-hydroxy T-2 triol, T-2 tetraol, and T-2 tetraol monoacetates, all mainly derived from T-2 toxin by either hydrolysis, deacylation, or hydroxylation of the isovalerate at C-8$\alpha$ position or/and by hydrolysis at C-4 and C-15 positions (Abbas et al., 1984; Gedek and Bauer, 1983; Marasas et al., 1984; Rabie et al., 1986).

The isolates of *F. acuminatum* of the present study differ from those previously reported mainly because 8-acetoxyneosolaniol (1), instead of T-2 toxin (having an acetate instead of an isovalerate group at C-8$\alpha$ position), is the major metabolite to which all the other trichothecenes are related. The production of compound 1 as well as other trichothecenes with an acetate at C-8$\alpha$, e.g. compounds **2**, **4**, **5**, and **7**, seems then to be a peculiarity of this group of isolates originated from corn stalk, ear, and soil in Italy. The use of such characteristics could be suggested as a chemotaxonomical marker of this particular type of *F. acuminatum*, although more isolates need to be investigated for toxigenicity in order to establish whether there is any relationship between host, substrate, or geographical origin and the type of trichothecene produced. Although *F. acuminatum* has not been associated with any human or animal toxicoses, we consider this species a very important one from the mycotoxicological point of view due to its widespread occurrence and the high toxicity and toxigenicity.

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