Isolation, Characterization, and Biological Activity of Visoltricin, a Novel Metabolite of *Fusarium tricinctum*

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A novel compound, trivially named visoltricin, has been isolated from cultures of *Fusarium tricinctum* on corn. Its structure has been established by chemical reactions and spectroscopic methods (UV, IR, MS, ^{1}H NMR, and ^{13}C NMR) as 3-[1-methyl-4-(3-methyl-2-butenyl)imidazol-5-yl]-2-propenoic acid methyl ester (molecular formula $C_{13}H_{18}N_2O_2$; molecular weight 234.297). It is the first imidazole derivative produced by a *Fusarium* species. About 30% of the tested isolates (33) of *F. tricinctum* from various substrates and geographic areas produced visoltricin when grown on corn. None of the 14 isolates tested from the United States produced the compound. Visoltricin was toxic to *Artemia salina* larvae ($LD_{50} = 8.5 \times 10^{-7}$ M) and cytotoxic ($IG_{50} < 10^{-5}$ M) to 6 human tumor cell lines (of 60 lines tested). Visoltricin induced on rabbit eye an interesting miotic effect which has been correlated to the anticholinesterase activity shown by the compound against human serum and pure enzymes.

Species of *Fusarium* are largely distributed in nature and produce a variety of toxic metabolites, such as trichothecenes, zearalenone, moniliformin, and fumonisins (Marasas *et al.*, 1984; Nelson *et al.*, 1991). One of the most controversial *Fusarium* species with respect to the taxonomy is *Fusarium tricinctum*, which has been related to cases of human and animal intoxications and has been reported erroneously in the literature as a trichothecene-producing species (Marasas *et al.*, 1984). A previous investigation of the production of trichothecenes by *Fusarium* species in relation to toxicity to *Artemia salina* larvae indicated the absence of trichothecenes in both toxic and nontoxic culture extracts of several strains of *F. tricinctum* (Corda) Sacc. (Logrieco *et al.*, 1990).

In this paper we report the isolation and chemical characterization of the major responsible compound for toxicity toward *A. salina* of cultures of *F. tricinctum*. The compound was identified by spectroscopic methods (UV, IR, ^{1}H and ^{13}C NMR, and MS) and chemical reactions as the 3-[1-methyl-4-(3-methyl-2-butenyl)imidazol-5-yl]-2-propenoic acid methyl ester and named "visoltricin" (structure in Figure 1) (Visconti and Solfrizzo, 1989). Data on the biological activity of visoltricin and the production by several strains of *F. tricinctum* isolated from various substrates and geographic areas are also reported.

EXPERIMENTAL PROCEDURES

Fungal Source and Culturing. *F. tricinctum* (Corda) Sacc. strain ITEM-649 (=KF-260), used for the large-scale production of visoltricin, was isolated from wheat kernels by J. Chelkowski (Agricultural University, Warsaw, Poland) and identified by A. Logrieco (Istituto Tossine e Micotossine, CNR, Bari, Italy) according to the classification system of Nelson *et al.* (1983). In addition to this, 31 strains of *F. tricinctum* from different sources (see Table 2) were tested for production of visoltricin on corn cultures.

Fungal strains were grown on 100 g of corn kernels brought up to about 45% moisture in 500-mL Erlenmeyer flasks and then autoclaved at 120 °C for 20 min. The substrate 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.

F. tricinctum strain ITEM-649 was also cultured on liquid media, such as YES (yeast extract sucrose), Czapek-Dox, and MYRO (Farber and Sanders, 1986). In particular, the fungus was grown under static conditions at room temperature for 2 weeks in the first medium and for 6 weeks in the latter two media.

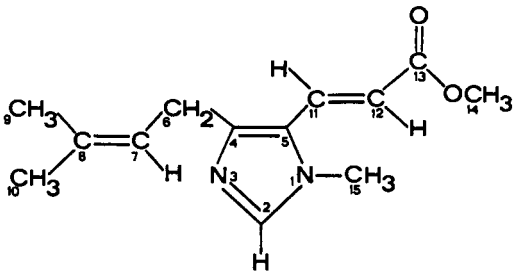

Figure 1. Chemical structure of visoltricin.

Production and Isolation of Visoltricin from *F. tricinctum* Strain ITEM-649. *F. tricinctum* strain ITEM-649 was grown (6 weeks at 25–27 °C) on 1.6 kg of corn kernels distributed in eight 1-L Erlenmeyer flasks. A first extraction was performed by shaking overnight with 3.4 L of MeOH–1% aqueous NaCl (55:45) in a rotary shaker. After filtration, the residue was re-extracted in a Waring Blendor with 2 L of the MeOH–NaCl solution. The filtrates were combined and concentrated to about 2 L in a rotary evaporator at 60 °C. The residue was defatted twice with 1 L of *n*-hexane and then extracted twice with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over anhydrous Na_2SO_4 and concentrated in a rotary evaporator. The residue (40 mL) was chromatographed on a 35-cm column (3 cm i.d.) packed with silica gel 60 in CH_2Cl_2. The column was eluted sequentially with 1000 mL of CH_2Cl_2, 500 mL of CH_2Cl_2–MeOH (98:2), 500 mL of CH_2Cl_2–MeOH (95:5), 500 mL of CH_2Cl_2–MeOH (90:10), and 500 mL of CH_2Cl_2–MeOH (80:20). Thirty 100-mL fractions were collected and tested for visoltricin by HPTLC. The compound was present in fractions 16–18, which were combined, then dried in a rotary evaporator, and dissolved in a minimum volume of acetonitrile. Aliquots (0.5 mL) of this solution were injected into the preparative HPLC apparatus, and 50 2.5-mL fractions (A1–A50) were collected for each run and tested for visoltricin by HPTLC. Visoltricin was present in fractions A41–A43. These fractions were combined, concentrated to dryness under vacuum, reconstituted with water, and lyophilized. The amount of visoltricin obtained with this procedure was 80 mg, with a purity degree higher than 98%. Purity was checked by analytical HPLC (see below) and HPTLC using the following solvent systems: (A) benzene–acetone (12:7), R_f = 0.37; (B) chloroform–methanol (9:1), R_f = 0.77; and (C) chloroform–methanol–1% acetic acid (90:10:1), R_f = 0.86.

An alternative procedure was used for the isolation of visoltricin from new culture batches, taking advantage of the basic nature of the compound. In particular, after evaporation of the MeOH from the MeOH–NaCl culture extract (obtained as described above), the solution containing visoltricin was acidified to pH 2

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with 37% HCl to form visoltricin hydrochloride. Then it was defatted twice with 0.5 volumes of *n*-hexane, and the organic interferences were extracted with 0.3 volumes of methylene chloride and discarded. Visoltricin was recovered from its salt in the water fraction by addition of 10 N NaOH up to pH 8 and extracted twice with 0.5 volumes of methylene chloride. After concentration to dryness, the residue was reconstituted in the minimum volume of MeOH and purified by semipreparative HPLC as reported above. The methylene chloride extract obtained with this procedure contained fewer interfering compounds and was easier to purify than the one obtained with the first procedure.

Chemical Reactions. Hydrolysis of visoltricin (1 mg) was perfomed by reaction with 0.1 N ethanolic NaOH (0.5 mL) for 4 h at 50 °C. The hydrolysis product was visualized by HPTLC as a blue spot (after spraying with *p*-anisaldehyde) at R_f values of 0.2 and 0.39 in solvent systems B and C, respectively. After dilution with water, the unreacted visoltricin was removed by extraction with CH_2Cl_2. The water phase was dried under vacuum, and the hydrolysis product was re-esterified with 0.5 mL of 14% methanolic BF_3 (20 min at 60 °C) to regenerate visoltricin. The latter was confirmed by HPTLC and GC/MS. The hydrolysis product reacted with Tri-Sil TBT (Pierce) to form the trimethylsilyl derivative which was detected by GC at longer retention time (14.3 min) with respect to visoltricin (unreacted, retention time 13.1 min).

To provide a water-soluble compound to be tested for miotic activity, the visoltricin hydrochloride was prepared by adding 0.1 N HCl to solid visoltricin in equimolar ratio. Appropriate dilutions were performed with distilled water prior to the assay.

Analysis of Visoltricin in Fungal Cultures. Visoltricin was extracted from corn cultures according to a procedure previously developed for other *Fusarium* toxins, i.e., trichothecenes and zearalenone (Bottalico *et al.*, 1983). In particular, 20 g of dried cultures was extracted with 100 mL of MeOH–1% aqueous NaCl (55:45) in a blender and filtered. The filtrates (50 mL) were defatted with *n*-hexane (2 × 25 mL) and partitioned with CH_2Cl_2 (3 × 25 mL). The residue after solvent evaporation was brought up to 1 mL with MeOH. Qualitative analyses of visoltricin were performed by HPTLC on silica gel precoated plates (10 × 10 cm) with fluorescence indicator, using benzene–acetone (12:7) and chloroform–methanol (9:1) as the solvent systems, which eluted the compound at R_f values of 0.37 and 0.77, respectively. Visoltricin was visualized on the plates by fluorescence quenching and confirmed by the blue color developed after the plates were sprayed with a *p*-anisaldehyde solution and heated at 110 °C for 5 min. Quantitative analyses of visoltricin were performed by HPLC after cleanup through a 500-mg Sep-Pak C_{18} minicolumn. In particular, 50 μL of methanolic extract, corresponding to 50 mg of culture material, was forced by aspiration through the minicolumn previously conditioned with 5 mL of MeOH and 5 mL of MeOH–H_2O (30:70). The column was washed with 2 mL of MeOH–H_2O (30:70), and visoltricin was eluted with 2 × 2 mL of MeOH–0.01% NH_4OH (80:20). Each sample, after solvent evaporation, was reconstituted in an appropriate amount of MeOH and analyzed by reversed-phase HPLC with UV detection. Liquid cultures (150 mL) were separated from the mycelium by filtration and lyophilized, then dissolved in 20 mL of 2 N HCl, and extracted with 10 mL of CH_2Cl_2 (discarded). After addition of 10 N NaOH to the aqueous phase up to pH 8, visoltricin was extracted with CH_2Cl_2 (2 × 10 mL) that was dried under vacuum. The residues were reconstituted with 0.5 mL of MeOH and analyzed by HPTLC and HPLC.

Instrumentation. *Analytical HPLC.* The HPLC apparatus was a Waters 625 LC system equipped with a Hewlett-Packard HP 1040 UV diode array detector connected to a HP 9000 Series 300 computer. The HPLC column was a RP-18 PLRP-S 5-μm 100 Å (150 × 4.6 mm) preceded by a PS-DVB guard cartridge (5 × 3 mm) with the same packing material (Polymer Laboratories Ltd., U.K.). The mobile phase was a mixture of acetonitrile–0.01% NH_4OH (35:65) at flow rate of 1 mL/min. Visoltricin was detected (UV absorption at 301 nm) at 8.2 min retention time. For quantitation peak heights were compared with a calibration curve obtained with a reference solution of visoltricin in methanol.

Semipreparative HPLC. A Perkin-Elmer Series 3B liquid chromatograph equipped with a Lichrosorb RP-18 column (250 × 10 mm) was used for the semipreparative HPLC with the following MeOH–water gradient (containing 0.01% NH_4OH) as the mobile phase: 35% MeOH for 5 min, then to 70% MeOH in 10 min, followed by 10 min at 70% MeOH.

Gas Chromatography/Mass Spectrometry. The GC/MS apparatus was a Hewlett-Packard Model 5995C equipped with a 12-m HP-1 cross-linked methylsilicone gum capillary column. The column temperature was programmed from 100 to 280 °C as follows: 3 min at 100 °C, then to 280 °C at 10 °C/min, then 5 min at 280 °C. A solid probe electron impact (EI) spectrum of visoltricin was obtained on a Finnigan MAT 4500 GC/MS system with an INCOS data system; the probe temperature was programmed from 40 to 350 °C. GC analyses of the trimethylsilyl derivatives were performed on a Hewlett-Packard Model 5830 equipped with a FID detector and a 10 m × 0.52 mm i.d. HP-1 wide-bore column with the same temperature program as the GC/MS.

Nuclear Magnetic Resonance. Proton and ^{13}C NMR spectra were carried out on a Varian XL-200 NMR spectrometer at 200 and 50.3 MHz, respectively, using $CDCl_3$ as solvent and TMS as calibrant. Confirmation of proton chemical shift assignments was made by the use of $^1H/^1H$ homonuclear correlation spectra (COSY) and by specific decoupling. Confirmation of ^{13}C chemical shift assignments was performed by the attached proton test (APT) and coupling constants.

UV, IR, and Elemental Analysis. The visoltricin UV spectrum (MeOH) was recorded on a Beckman DU-65 spectrophotometer. The IR spectrum (KBr tablet) was performed on a Fourier transform spectrometer (Perkin-Elmer Model 2000). A Carlo Erba Model 1106 apparatus was used for the elemental analysis.

Biological Activity. *A. salina Assay.* The *A. salina* bioassay (brine shrimps) was carried out as reported elsewhere (Visconti *et al.*, 1989).

Cytotoxic Activity. Visoltricin was submitted to the National Cancer Institute (NCI) preclinical antitumor drug discovery screening program, i.e., the disease-oriented, *in vitro* primary screen employing a human tumor cell line panel (Boyd, 1989). This involved the *in vitro* testing of visoltricin at five concentrations at 10-fold dilution toward a panel of 60 human tumor cell lines, derived from seven cancer types (lung, colon, melanoma, renal, ovarian, brain, and leukemia) (Boyd, 1989). Visoltricin was also tested for the inhibition of PHA-stimulated human lymphocyte proliferation by using the MTT assay reported elsewhere (Visconti *et al.*, 1991, 1992).

Miotic Activity. New Zealand rabbits weighing 2–2.5 kg were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar). Distilled water and 1–2% pilocarpine hydrochloride (Allergan S.p.A.) were used as the negative and positive controls, respectively. Visoltricin was applied as 2% visoltricin hydrochloride solution (4 drops) on the left eye and compared with the control (positive or negative), which was applied on the right eye of the same animal. Triplicate experiments were performed. Atropine sulfate (0.5–1%, Allergan) was added to test the reversibility of the miotic effect (pupil diameter decrease).

RESULTS AND DISCUSSION

Characterization of Visoltricin. *General Characteristics.* Visoltricin appeared as a white powder with a molecular weight of 234.297 (confirmed by MS) and a melting point of 93 °C. Elemental analysis gave the following results, in agreement with the molecular formula $C_{13}H_{18}N_2O_2$: C, 66.79% (calcd 66.64%); H, 7.71% (calcd 7.74%); N, 11.91% (calcd 11.96%); O, 13.59% (calcd 13.66%). Visoltricin was soluble (3% w/v) in methylene chloride, chloroform, acetone, benzene, tetrahydrofuran, and 0.1 N hydrochloric acid, less soluble (1% w/v) in methanol, ethyl acetate, and acetonitrile, and insoluble in *n*-hexane, ethyl ether, and water.

Spectral Data. The following spectral data were recorded for visoltricin: UV, λ_{max} = 300 nm, ϵ = 20.304 (log ϵ = 4.31); IR, ν_{max}, 3097, 2980–2820, 1704, 1633, 1520,

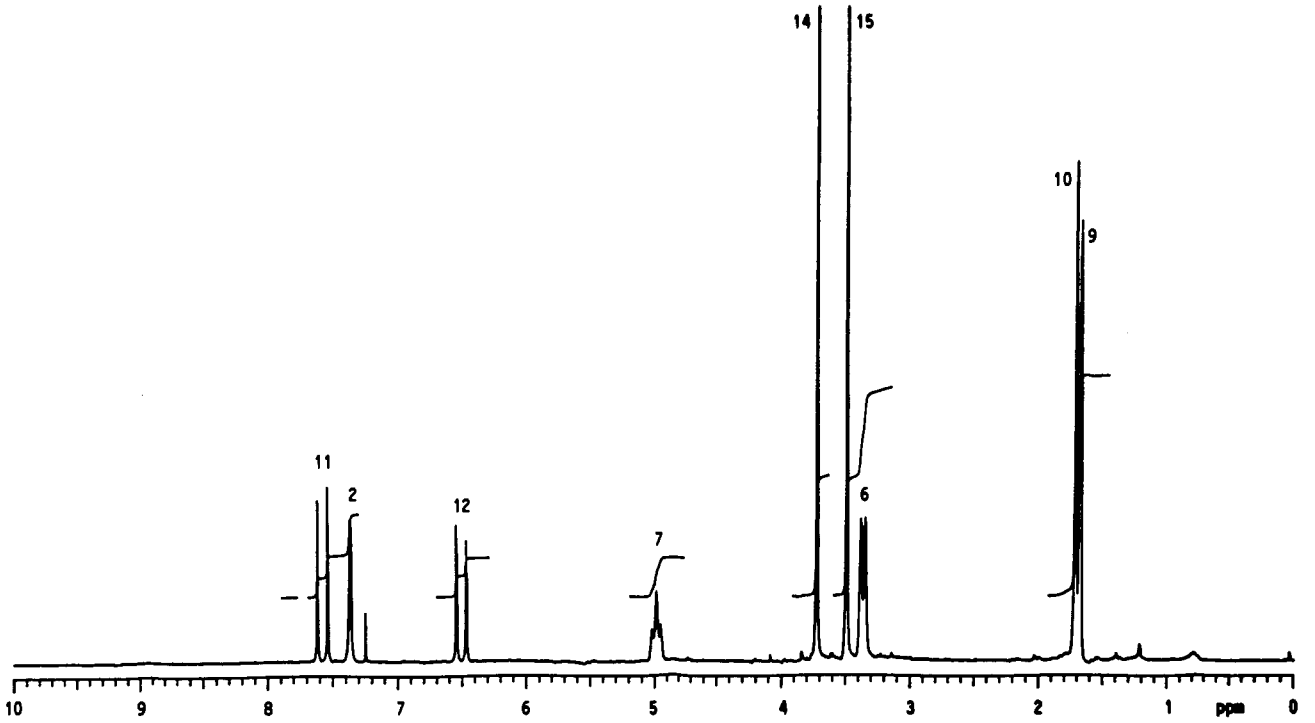

Figure 2. Proton NMR spectrum of visoltricin in $CDCl_3$.

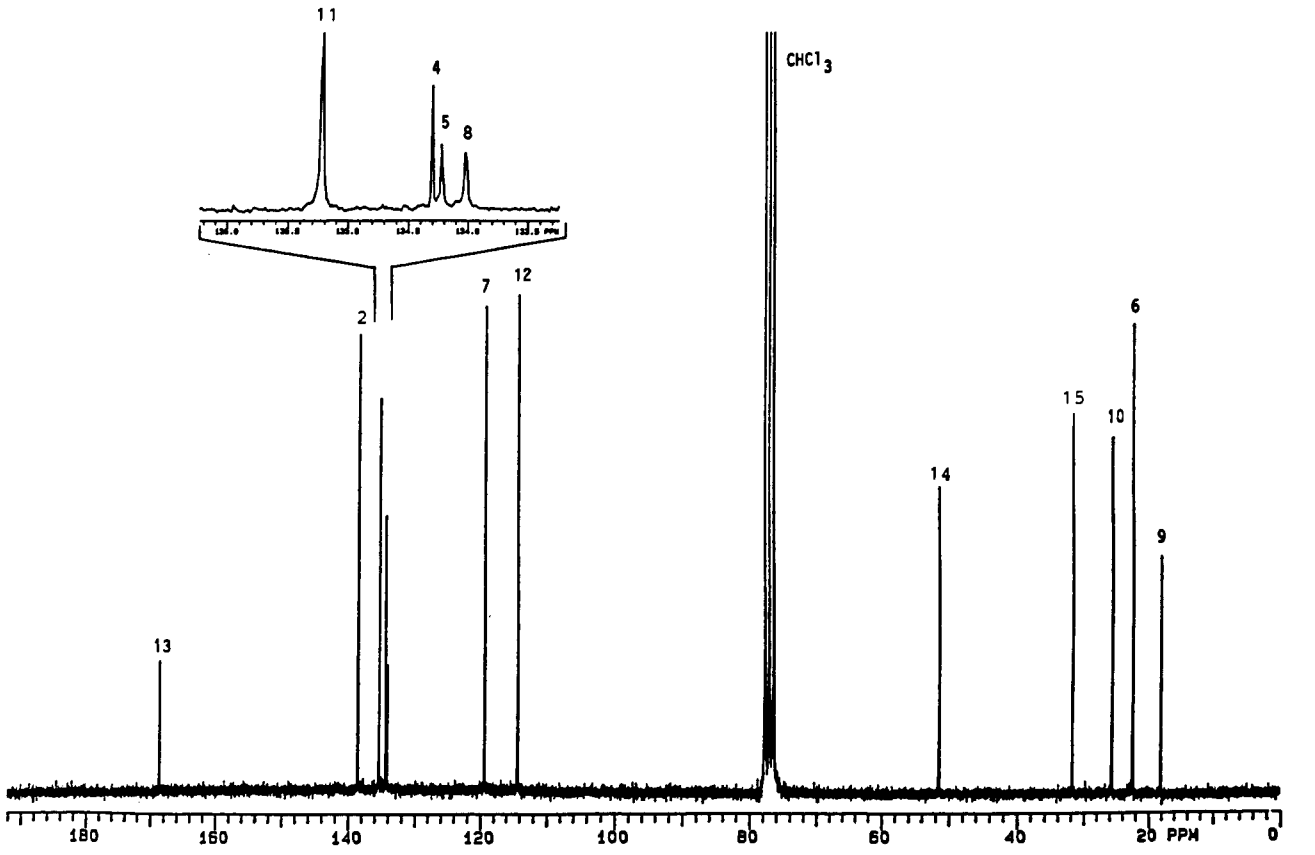

Figure 3. ^{13}C NMR spectrum of visoltricin in $CDCl_3$.

1463, 1433, 1384, 1304, 1276, 1166, 1000, 869, 803 cm^{-1}; MS, *m/z* (relative intensity) 234 (43, M^+), 219 (2), 202 (91), 187 (21), 173 (64), 159 (100), 133 (52); NMR, ^1H and ^{13}C NMR spectra are illustrated in Figures 2 and 3, and the relevant chemical shifts and coupling constants are reported in Table 1.

The ester C=O and the conjugated double bond were evident in the IR spectrum by the signals at 1705 and 1633 cm^{-1}. Signals relevant to *trans–trans* conjugated alkene (nontetrasubstituted) were observed at 3097, 1633, 1000, and 803 cm^{-1}. Typical signals of methylimidazole were observed at 1520 and 1276 cm^{-1}.

The following information was derived from the mass spectrum. The loss of a methyl group from the molecular ion (*m/z* 234) gave rise to the fragment at *m/z* 219. The latter generated the fragments at *m/z* 187 ($-CH_3OH$), 133

Table 1. Chemical Shift (δ) and Coupling Constant (J in Hertz) Assignments in Visoltricin ^1H and ^{13}C NMR Spectra

position	^1H	^{13}C
2	1H 7.37s	138.4d[a] (J = 207.3)
4		134.3
5		134.2
6	2H 3.39d[b] ($J_{6,7}$ = 6.8)	22.3t[a] (J = 127.5)
7	1H 4.99m[b] ($J_{7,6}$ = 6.8)	119.3m
8		134.0m
9	3H 1.68[b] ($J_{9,10}$ = 1.3)	17.9q (J = 125.5)
10	3H 1.71[b] ($J_{10,9}$ = 1.3)	25.5q (J = 125.2)
11	1H 7.60d ($J_{11,12}$ = 15)	135.2d (J = 153.4)
12	1H 6.51d ($J_{12,11}$ = 15.5)	114.2d[a] (J = 164.9)
13		168.5
14	3H 3.73s	51.4q (J = 146.4)
15	3H 3.50s	31.6q (J = 140.0)

[a] Long-range coupling constant observed in C-2 (^2J = 3.5), C-6 (^2J = 4.3), C-12 (^2J = 3.7). [b] Split signals with long-range coupling constant of 1.3 Hz.

(by the loss of the methyl acrylate substituent at the C-5 position of the heterocycle), and 159 (- CH_3OH, - CO; base peak). The loss of methanol from the molecular ion generated the signal at m/z 202.

The ^1H NMR and ^{13}C NMR chemical shift and coupling constant assignments were in good agreement with the calculated values (Silverstein *et al.*, 1974; Stothers, 1972) and confirmed by appropriate spin–spin decoupling and COSY experiments. In the ^1H NMR spectrum the high coupling constants of C-11 and C-12 protons indicated a *trans* configuration at the relevant double bond. The downfield signal of the C-2 proton (broad singlet) and the three-proton singlet at 3.50 ppm (N–CH_3) were typical of *N*-methylimidazole. The long-range couplings of C-6, C-7, C-9, and C-10 were in good agreement with the assigned structure. ^{13}C NMR chemical shifts and ^{13}C–^1H coupling constants of substituted *N*-methylimidazole reported in the literature (Pachler *et al.*, 1981) are in good agreement with visoltricin assigned structure. Moreover, the ^{13}C APT (attached proton test) confirmed the above assignments, showing in particular eight mono- or trisubstituted (C-2, C-7, C-9, C-10, C-11, C-12, C-14, and C-15), and five di- or tetrasubstituted (C-4, C-5, C-6, C-8, and C-13) carbon atoms.

On the basis of the above spectroscopic data and chemical reactions the structure of visoltricin was established as 3-[1-methyl -4-(3-methyl-2-butenyl)imidazol-5-yl]-2-propenoic acid methyl ester [*synonyms:* 5-(1-methyl-4-prenyl)imidazoleacrylic acid methyl ester or *N*-methylprenylurocanic acid methyl ester].

Production of Visoltricin by *F. tricinctum* Strains. The production of visoltricin by different strains of *F. tricinctum* isolated from various substrates and geographical areas is reported in Table 2. About 30% of the tested strains (10 of 32) were able to produce visoltricin at levels ranging from 20 to 1350 mg/kg. The production of visoltricin was not affected by the substrate of origin, as the compound was produced by strains isolated from wheat, barley, oats, *Trifolium pratense*, mulberry, onion bulb, and soil. In contrast, a role seemed to be played by the geographic area from which the fungus originated, as indicated by the complete absence of visoltricin producers within the 14 strains isolated in the United States. A considerable number of visoltricin-producing strains (10 of 18) were found instead within the isolates originating from other countries, such as Poland, Italy, Hungary, Germany, Sweden, Denmark, China, and Australia.

The average visoltricin concentration (based on several experiments performed in different periods) found in corn cultures of *F. tricinctum* ITEM-649 was 100 μg/g. Visoltricin was also formed in Czapek-Dox, YES, and MYRO liquid cultures of the same fungus, as shown by the detection of the compound in the culture filtrates at concentrations of 1.5, 1.0, and 0.15 mg/L, respectively. Although the possibility that in liquid cultures visoltricin accumulates in the mycelium cannot be ruled out, these levels are very low compared to those found in cultures on solid corn. The latter resulted in a more appropriate substrate for large-scale production of visoltricin. A preliminary experiment on the time course of visoltricin production (at 25–27 °C on sterilized corn) showed that production starts after 2 weeks and then increases until the maximum production plateau is reached after 5 weeks. An incubation time of 6 weeks was then established as optimal for large-scale visoltricin production, whereas a 4-week incubation provided a good time–production compromise for screening several strains.

Table 2. Production of Visoltricin by Strains of *F. tricinctum* Isolated from Various Substrates and Geographic Areas

strain	substrate of origin	geographic area	visoltricin content (μg/g)
ITEM-649 (KF-260)	wheat kernel	Poland	100
ITEM-648	wheat kernel	Poland	30
ITEM-650 (KF-259)	wheat kernel	Poland	nd[b]
KF-105	barley	Poland	nd
KF-106	barley	Poland	nd
KF-107	rye	Poland	nd
B-3	wheat kernel	Italy	200
B-8	wheat kernel	Italy	nd
T-206	corn	Yugoslavia	nd
T-226	onion bulb	Hungary	40
T-387	*T. pratense*	Germany	29
T-388	wheat kernel	Finland	nd
T-511	mulberry stem	Australia	271
T-545	pasture soil	Australia	30
T-577	sorghum stalk	Australia	nd
T-693	soil	China	1350
T-823	barley ear	Denmark	22
T-904	oats	Sweden	1165
T-79	carnation stub	Pennsylvania	nd
T-171	corn stalk	Pennsylvania	nd
T-263	soil sample	Mississippi	nd
T-274	wheat	Kansas	nd
T-276	wheat	Nebraska	nd
T-289	barley	Idaho	nd
T-323	wheat	Ohio	nd
T-325	wheat	Indiana	nd
T-329	rye	Minnesota	nd
T-367	barley	South Dakota	nd
T-371	barley	North Dakota	nd
T-382	watermilfoil	Wisconsin	nd
T-460	birdsfoot trefoil	New York	nd
T-638	corn stalk	Minnesota	nd

[a] Accession numbers to the following collections: ITEM, Istituto Tossine e Micotossine, CNR, Bari, Italy; KF, Katedra Fitopatologii, Agricultural University, Warsaw, Poland; T, *Fusarium* Research Center, Pennsylvania State University, University Park, PA; B, isolated by C. Sabia (ITEM). [b] Not detected (<1 μg/g).

Biological Activity of Visoltricin. Visoltricin was toxic to *A. salina* larvae with an LD_{50} value of 0.2 μg/mL (8.5×10^{-7} M), a level similar to that of the most toxic *Fusarium* trichothecenes (Visconti *et al.*, 1992).

Some cytotoxic activity resulted for visoltricin from the NCI screening of 60 human tumor cell lines. In particular, three of six leukemia lines (HL-60TB, K-562, and MOLT-4), two of nine colon cancer lines (HCT-15 and KM12), and one of two small-cell lung cancer lines (DMS114) showed a 50% growth inhibition (IG_{50}) ranging from 10^{-6} to 10^{-5} M. Visoltricin did not inhibit lymphocyte proliferation up to concentration of 2×10^{-5} M.

Visoltricin is the first imidazole derivative produced by a *Fusarium* species. The structure of visoltricin contains an *N*-methylimidazole group which is also present in the molecule of pilocarpine, a cholinergic drug largely used as a miotic agent in the therapy of glaucoma. For this reason visoltricin was tested for miotic activity (pupil diameter decrease) toward rabbit eye, inducing an effect with intensity corresponding to about 70% of that of pilocarpine. The miotic effect of visoltricin could be observed after 20 min from the treatment and for a duration of 4–6 h. Atropine sulfate behaved as an antagonist for both pilocarpine and visoltricin, giving rise to the complete neutralization of the miotic effect. Unlike pilocarpine, visoltricin did not show any muscarinic effect when tested against guinea pig ileum (M. Mazzoccoli, personal communication). It showed instead anticholinesterase activity toward human serum and pure mammalian enzymes. Kinetics studies showed for visoltricin a mixed-type and reversible inhibition of the EC 3.1.1.7 cholinesterase enzyme with $K_i = 1.9 \times 10^{-4}$ M (Solfrizzo and Visconti, 1993). This activity, even though low in intensity, could explain at least partially the miotic effect on rabbit eye. A detailed study of visoltricin anticholinesterase activity and its correlation with the miotic activity is reported elsewhere (Solfrizzo and Visconti, 1993).

It will be interesting to test visoltricin or its derivatives for their potential application in medicine in the therapy of glaucoma or of other diseases which can be treated with anticholinesterase agents. However, the most immediate need is to establish a relationship between visoltricin and toxic effect in mammals, for which further investigations are necessary. The moderate cytotoxic and anticholinesterase activity of visoltricin and its strong toxicity to *A. salina* (although the correlation between brine shrimp lethality and mammalian toxicity has not been proved) suggest that particular safety precautions must be taken in handling this compound and *Fusarium* cultures that may contain it.

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