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Bioconversion of Possible T-2 Toxin Precursors by a Mutant Strain of *Fusarium sporotrichioides* NRRL 3299

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Liquid cultures of a mutant strain of *Fusarium sporotrichioides* NRRL 3299 that accumulates trichodiene rather than T-2 toxin converted tricho-9-ene-2α,3α,11α-triol, trichotriol (tricho-10-ene-2α,3α,9α-triol), tricho-10-ene-2α,3α,9β-triol, 3α-hydroxytrichothecene, and 3α-acetoxytrichothecene to T-2 toxin. Other possible oxygenated precursors of T-2 toxin, including trichodiol (tricho-10-ene-2α,9α-diol), trichothecene, 4α-hydroxytrichothecene, and 15-hydroxytrichothecene, were not metabolized. The results indicate that in the biosynthesis of T-2 toxin by *F. sporotrichioides*, (i) oxygenation at C-3 occurs prior to the second cyclization, (ii) this second cyclization involves two steps that may be nonenzymatic, and (iii) oxidation at C-3 precedes that at C-4 or C-15.

Trichothecenes are sesquiterpenoid metabolites produced by several groups of fungi, including *Fusarium*, *Trichothecium* and *Myrothecium* species. Considerable variation exists in the oxygenation pattern of individual toxins, but all are characterized by a double bond and a 12,13-epoxide group. The biosynthesis of trichothecenes proceeds from the alicyclic hydrocarbon trichodiene (10, 19), which is a cyclization product of farnesyl pyrophosphate (10). The conversion of trichodiene (compound 15; see Fig. 5) to T-2 toxin (compound 16; see Fig. 5), the major trichothecene produced by *Fusarium sporotrichioides*, is known to involve six oxygenations with molecular oxygen (6) and a second cyclization, but specific oxygenated intermediates have not been identified.

Blocked mutant strains of toxigenic fungi have been useful tools in biosynthetic studies of mycotoxins. For instance, bioconversion studies with nonaflatoxigenic strains of *Aspergillus parasiticus* identified a series of anthraquinones, isolated from wild-type and mutant strains of *A. parasiticus*, as precursors of aflatoxin (2, 11, 13). In order to do analogous studies on trichothecene biosynthesis, stable mutant strains, blocked in T-2 toxin biosynthesis, have been produced via UV irradiation of a T-2 toxin-producing strain, *F. sporotrichioides* NRRL 3299 (3, 14).

In the course of analyzing these *F. sporotrichioides* mutant strains, several novel trichothecenes with a tricho-9-ene-11α-hydroxyl skeleton (compounds 8 through 11; see Fig. 2 and Table 1) were isolated and identified (14). Compounds of this type have been proposed to be involved in the biosynthesis of both sambucoins and apotrichothecenes (1, 15). Their accumulation by mutant strains of *F. sporotrichioides* deficient in their ability to synthesize T-2 toxin suggests that they may also play a role in trichothecene biosynthesis.

In order to determine whether these new metabolites have a role in the trichothecene pathway and whether there is an ordered sequence of cyclization and oxygenation, these new compounds and a number of other possible precursors of T-2 toxin were tested for their ability to be converted to T-2 toxin by *F. sporotrichioides* MB5493. In liquid culture, this mutant strain accumulates the hydrocarbon trichodiene rather than T-2 toxin (M. N. Beremand, P. J. Black, and R. D. Plattner, J. Cell. Biochem. Suppl. **12c**:261, 1988) and is therefore well-suited for feeding possible oxygenated precursors of T-2 toxin.

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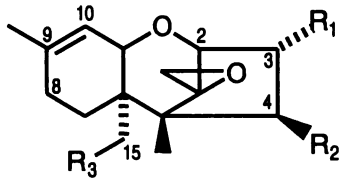
MATERIALS AND METHODS

Physical analyses. Thin-layer chromatography (TLC) was done on silica gel 60 F-254 plates (5 by 10 or 20 by 20 cm, 0.25 mm thick; E. Merck AG, Darmstadt, Federal Republic of Germany) with CH_2Cl_2-methanol (MeOH) (9:1) or ethyl acetate-MeOH (9:1), unless otherwise noted. Chromatograms were visualized under a short-wavelength UV lamp and with two spray detection systems: (i) a 3% solution of ceric sulfate in 3 N sulfuric acid, which gives brown spots on a white background after heating; or (ii) 4-(*p*-nitrobenzyl)pyridine and tetraethylenepentamine, which gives a blue spot on a white background in compounds containing an epoxide moiety (8). High-pressure liquid chromatography separations were carried out on an SP8100 instrument (Spectra-Physics, San Jose, Calif.) fitted with a Microsorb silica column (5 μm of silica; 25-cm length by 4.6-mm inside diameter) (Rainin Instrument Co., Woburn, Mass.) with a 2-propanol-hexane (3:7) solvent system at a flow rate of 1 ml/min and detection at 210 nm. Gas chromatography (GC) measurements of trimethylsilyl (TMS) ethers were made by flame ionization detection on a Spectra-Physics SP7100 chromatograph fitted with a 30-m fused-silica capillary column (DB1; 0.25 μm) (J&W Scientific, Folsom, Calif.). The column was held at 120°C at injection; it was then heated to 210°C at 15°C/min and held for 1 min and then heated to 260°C at 5°C/min and held for 8 min. Low-resolution mass spectra were obtained on a mass selective detector (model 5979; Hewlett Packard Co., Palo Alto, Calif.), with the ion source operated at 250°C and 70 eV ionization energy. Samples were introduced to the mass selective detector through a gas chromatographic inlet with a cross-linked dimethyl silicone fused-silica column (12.5 m by 0.2 mm) in the splitless mode at 120°C. The column was held isothermal for 2 min and then programmed to 200°C at 40°C/min, followed by slower heating at 10°C/min to 270°C. It was then held isothermal for 4 min. The injector was heated at 250°C.



	R_1	R_2	R_3
1	H	H	H
2	OH	H	H
3	OAc	H	H
4	H	OH	H
5	H	OAc	H
6	H	H	OH
7	H	H	OAc

FIG. 1. Structures of trichothecene (compound 1) and monohydroxy- and monoacetoxytrichothecenes, 3α-hydroxytrichothecene (compound 2), 3α-acetoxytrichothecene (compound 3), 4β-hydroxytrichothecene (compound 4), 4β-acetoxytrichothecene (compound 5), 15-hydroxytrichothecene (compound 6), and 15-acetoxytrichothecene (compound 7).

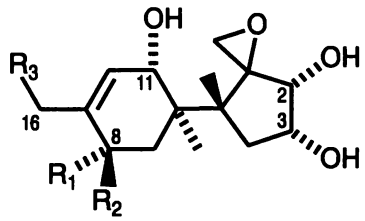
TMS ethers were prepared with Tri-Sil/TBT, a formulation of TMS-imidazole, bis-TMS-acetamide, and trimethylchlorosilane (Pierce Chemical Co., Rockford, Ill.), and dissolved in hexane.

Trichothecenes for feeding. Trichothecenes were isolated from *Trichothecium roseum* (no. A27955) or from mutant strains of *F. sporotrichioides* NRRL 3299 (3, 14) or were derived synthetically from available compounds. All reagents were from Aldrich Chemical Co., Inc., Milwaukee, Wis., unless otherwise noted. Structure identifications were confirmed by mass spectrometry and proton nuclear magnetic resonance (NMR). Prior to feeding, purity was checked by TLC with both spray detection systems and by GC of the TMS ether. All compounds were greater than 95% pure by GC.

Trichodiol (tricho-10-ene-2α,9α-diol; compound 12; see Fig. 3) and trichodermol (4β-hydroxytrichothecene; compound 4; see Fig. 1) were isolated from liquid shake cultures of *T. roseum*. Cultures (1 liter of yeast extract–peptone–dextrose [YEPD] in 2.8-liter Fernbach flasks) were extracted three times with ethyl acetate (1 liter). The concentrated extract was separated by preparative silica gel TLC (5% MeOH-CH_2Cl_2). Trichodermin (4β-acetoxytrichothecene; compound 5) was prepared by stirring trichodermol overnight in a 1:1 mixture of pyridine and acetic anhydride.

Isotrichodermol (3α-hydroxytrichothecene; compound 2; Fig. 1), tricho-9-ene-2α,3α,11α-triol (compound 8; Fig. 2), tricho-9-ene-2α,3α,8α,11α-tetraol (compound 9; Fig. 2), tricho-9-ene-2α,3α,8β,11α-tetraol (compound 10; Fig. 2), tricho-9-ene-2α,3α,11α,16-tetraol (compound 11; Fig. 2), and tricho-10-ene-2α,3α,9α-triol (compound 13; Fig. 3) were all isolated from *F. sporotrichioides* MB2972 as described previously (14). Isotrichodermin (3α-acetoxytrichothecene; compound 3; Fig. 1) was prepared by stirring isotrichodermol overnight with a 1:1 mixture of acetic anhydride and pyridine.

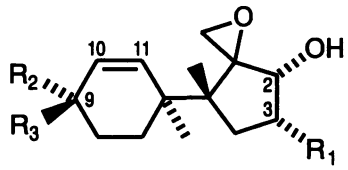
Tricho-10-ene-2α,3α,9β-triol (compound 14; Fig. 3) was isolated from a solid rice culture of *F. sporotrichioides* MB2972 which had been extracted and partitioned as described previously (14). Separation on analytical high-pressure liquid chromatography with 2-propanol–hexane (3:7) resulted in the isolation of tricho-10-ene-2α,3α,9α-triol (11.0 min; compound 13; Fig. 3) and tricho-10-ene-2α-3α,9β-triol (11.8 min; compound 14; Fig. 3).



	R_1	R_2	R_3
8	H	H	H
9	OH	H	H
10	H	OH	H
11	H	H	OH

FIG. 2. Structures of modified 11-hydroxytrichothecenes, tricho-9-ene-2α,3α,11α-triol (compound 8), tricho-9-ene-2α,3α,8α,11α-tetraol (compound 9), tricho-9-ene-2α,3α,8β,11α-tetraol (compound 10), and tricho-9-ene-2α,3α,11α,16-tetraol (compound 11).

Trichothecene (compound 1; Fig. 1) was prepared from 3α-hydroxytrichothecene by a two-step Barton-McCombie deoxygenation (18). A solution of 48.2 mg of 3α-hydroxytrichothecene, 25.5 mg of 4-dimethylaminopyridine, and 73.1 μl of pyridine in 25 ml of dry methylene chloride was treated with 63.4 μl of phenylchlorothionocarbonate. The mixture was stirred at room temperature under nitrogen for 48 h. The reaction mixture was concentrated and separated by preparative TLC (1% MeOH-CH_2Cl_2) to yield 33.8 mg of the phenylthiocarbonate and 14.0 mg of unreacted 3α-hydroxytrichothecene. The phenylthiocarbonate was heated to reflux with 16 mg of 2,2-azobis(2-methylpropanenitrile) (AIBN; Eastman Kodak Co., Rochester, N.Y.) in 25 ml of dry benzene. Tri-*n*-butyltinhydride (320 μl) was added, and heating under nitrogen was continued overnight. The reaction mixture was concentrated to dryness on a rotary evaporator, redissolved in 5% aqueous acetonitrile and extracted with hexane. The acetonitrile layer was dried over Na_2SO_4 and concentrated to dryness. Preparative TLC (1% MeOH-CH_2Cl_2) of the product resulted in isolation of trichothecene (5.3 mg).



	R_1	R_2	R_3
12	H	OH	H
13	OH	OH	H
14	OH	H	OH

FIG. 3. Structures of tricho-10-ene-2α,9α-diol (trichodiol; compound 12), tricho-10-ene-2α,3α,9α-triol (trichotriol; compound 13), and tricho-10-ene-2α,3α,9β-triol.

15-Acetoxytrichothecene (compound 7; see Fig. 1) was prepared from diacetoxyscirpenol (18), which had been isolated from *F. sporotrichioides* MB1716 (3). Diacetoxyscirpenol was mixed with dilute NaOH for 5 min to yield 15-monoacetoxyscirpenol (21 mg), which was then mixed with 60 μl of pyridine and 20 mg of 4-dimethylaminopyridine in 15 ml of CH_2Cl_2. Phenylchlorothionocarbonate (60 μl) was added, and the mixture was stirred under nitrogen at room temperature overnight. The reaction mixture was concentrated and cleaned by preparative TLC (10% MeOH-CH_2Cl_2). The resulting bis-phenylthiocarbonate (16.7 mg) was heated to reflux with 8 mg of AIBN in 25 ml of dry benzene. Tri-*n*-butyltinhydride was added, and the mixture was refluxed overnight. The reaction mixture was evaporated to dryness, and the residue was redissolved in 5% aqueous acetonitrile and then washed with petroleum ether. The acetonitrile layer was dried over Na_2SO_4, concentrated, and cleaned by preparative TLC with hexane-ethyl acetate (1:1) to yield 10.9 mg of 15-acetoxytrichothecene. 15-Hydroxytrichothecene (compound 6; Fig. 1) was prepared by treating 15-acetoxytrichothecene with dilute NaOH.

Feeding protocol. *F. sporotrichioides* MB5493 cultures were initially grown on V-8 agar plates (3) for 7 days. Liquid cultures were inoculated with conidia washed from the V-8 plates, at a starting density of 5×10^4 conidia per ml of YEPD-5G media (3), and incubated on a rotary shaker (200 rpm) at 28°C. After 4 days, a 5- or 10-ml sample of the culture was transferred to a 25-ml Erlenmeyer flask; a 25 mM stock solution of the test compound in dimethyl sulfoxide was added to the culture at a final concentration of 250 μM. Cultures were incubated on the rotary shaker at 28°C for an additional 4 days and then analyzed as described below.

Nonenzymatic interconversions. The pH of the media was measured prior to inoculation (Digital Ionalyzer 601A; Orion Research, Inc., Cambridge, Mass.). The pH of the culture filtrate was measured after 7 days of growth. In order to determine the role of pH in the conversion of the trichotriols, YEPD-5G media was adjusted to pH 7.3 or 6.5 with dilute NaOH and to pH 5.7, 5.2, 4.7, or 4.1 with dilute HCl, and 10 ml portions of each were placed in 25-ml Erlenmeyer flasks. A 25 mM solution of tricho-9-ene-2α,3α,11α-triol (compound 8; see Fig. 2) in dimethyl sulfoxide was added to each treatment to a final concentration of 250 μm. The flasks were incubated at 28°C on a rotary shaker (200 rpm) for 7 days and then analyzed as described below.

Extraction and analysis of samples. A 1-ml sample of the culture or the pH-adjusted media was extracted two times with 2 ml of ethyl acetate by mixing on a vortex. The combined ethyl acetate extracts were dried under a stream of nitrogen and resuspended in 1 ml of ethyl acetate. A 250-μl portion was removed, dried under nitrogen, derivatized with 100 μl of TBT, and dissolved in 150 μl of hexane. A 1-μl portion of the TMS-derivatized sample was analyzed by GC or GC-mass spectrometry (Fig. 4). The limit of detection of the trichothecenes analyzed by GC-mass spectrometry was 5 to 10 ng/μl.

RESULTS

Characterization of tricho-10-ene-2α,3α,9β-triol (compound 14; Fig. 3). The electron impact mass spectrum of the TMS ether of compound 14 exhibited an M-15 fragment ion at *m/z* 469, consistent with an uncyclized trichothecene with 3 trimethylsilyl groups. A fragment ion at *m/z* 169 resulted from retro-Diels-Alder cleavage of the A-ring. Mass spectral fragmentation of compound 14 was identical to that of tricho-10-ene-2α,3α,9α-triol (compound 13). Analysis by GC of the TMS ether of compound 14 resulted in a single peak at 12.81 min; the TMS ether of tricho-10-ene-2α,3α,9α-triol eluted at 12.43 min. The ^1H NMR spectrum differed from that of trichotriol primarily in the shifts of the C-13 protons and in the shifts and coupling constants of the C-10 and C-11 protons. The ^{13}C NMR spectra of the two triols showed significantly different shifts in the signals for the C-7, C-9, C-10, C-11, and C-16. The shifts and the coupling constants of the H-2, H-3, and H-4 signals were essentially the same for both compounds, which suggested that only the orientation of the C-9 hydroxyl group was reversed. Compound 14 was therefore identified as tricho-10-ene-2α,3α,9β-triol.

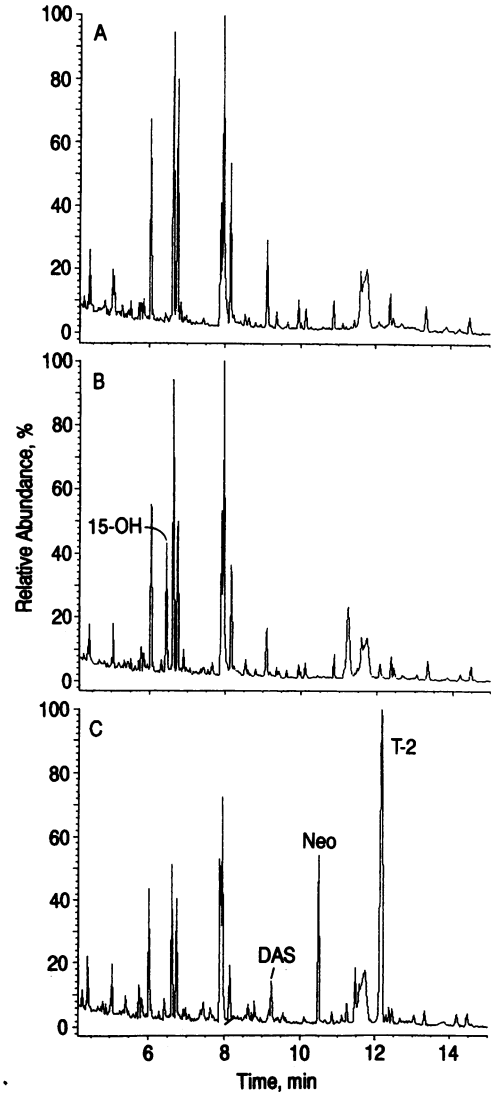


FIG. 4. Representative gas chromatograms of ethyl acetate extracts (after derivatization with TriSil/TBT) of *F. sporotrichioides* MB5493 cultures incubated for 4 days with dimethyl sulfoxide (A), 15-hydroxytrichothecene (15-OH; compound 6) (B), or tricho-10-ene-2α,3α,9α-triol (compound 13) (C). Chromatogram C indicates that compound 13 has been bioconverted to diacetoxyscirpenol, (DAS), neosolaniol (NEO), and T-2 toxin (T-2).

Feeding monohydroxytrichothecenes and monoacetoxytrichothecenes to MB5493. In order to determine whether there was an ordered sequence of oxygenation, six different mono-

TABLE 1. Results of feeding possible T-2 toxin precursors to *F. sporotrichioides* MB5493

Compound no.	Compound fed	Appearance of T-2 toxin[a]
1	Trichothecene	−
2	3α-Hydroxytrichothecene (isotrichodermol)	+
3	3α-Acetoxytrichothecene (isotrichodermin)	+
4	4β-Hydroxytrichothecene (trichodermol)	−
5	4β-Acetoxytrichothecene (trichodermin)	−
6	15-Hydroxytrichothecene	−
7	15-Acetoxytrichothecene	−
8	Tricho-9-ene-2α,3α,11α-triol	+
9	Tricho-9-ene-2α,3α,8α,11α-tetraol	−
10	Tricho-9-ene-2α,3α,8β,11α-tetraol	−
11	Tricho-9-ene-2α,3α,11α,16-tetraol	−
12	Tricho-10-ene-2α-9α-diol (trichodiol)	−
13	Tricho-10-ene-2α,3α,9α-triol (trichotriol)	+
14	Tricho-10-ene-2α,3α,9β-triol	+

[a] Nonconversion (−) indicates that T-2 toxin was not detected by GC-mass spectrometry at the 5- to 10-ng/μl level and that the trichothecene fed was detected in the culture extract. Conversion (+) indicates that T-2 toxin was detected in the culture extract; on a molar basis, the percent conversion to T-2 toxin ranged from 30 to 60%.

hydroxy- or monoacetoxytrichothecenes were fed to *F. sporotrichioides* MB5493. The results are summarized in Table 1. Only 3α-hydroxytrichothecene (compound 2; Fig. 1) and 3α-acetoxytrichothecene (compound 3; Fig. 1) were converted to T-2 toxin. T-2 toxin was not detected in cultures fed the other four trichothecenes, 4β-hydroxytrichothecene (compound 4; Fig. 1), 4β-acetoxytrichothecene (compound 5; Fig. 1), 15-hydroxytrichothecene (compound 6; Fig. 1), and 15-acetoxytrichothecene (compound 7; Fig. 1); in each case, the particular trichothecene fed was detected in the culture extract. This suggests that there is an ordered sequence of oxygenation and that oxygenation at C-3 precedes that at C-4 or C-15. The addition of an acetyl group did not alter the bioconversion of the trichothecenes with hydroxyls at C-3, C-4, or C-15.

Feeding trichodiol, trichothecene, and trichotriol to MB5493. Trichodiol (compound 12; Fig. 3) and trichothecene (compound 1; Fig. 1) were previously shown to be precursors of trichothecin (4β-butyryloxy-trichothecen-8-one), which is the major mycotoxin produced by *T. roseum* (11). Feeding these compounds to MB5493 resulted in no bioconversion to T-2 toxin. Tricho-10-ene-2α,3α,9α-triol (compound 13; Fig. 3) and its isomer, tricho-10-ene-2α,3α,9β-triol (compound 14; Fig. 3) were both converted to T-2 toxin by *F. sporotrichioides* MB5493. MB5493 was not able to oxygenate trichothecene at C-3. Since both 3α-hydroxytrichothecene and 3α-acetoxytrichothecene were converted to T-2 toxin, the results suggest that oxidation at C-3 occurs prior to cyclization in *F. sporotrichioides*.

Feeding 9-ene-11α-hydroxy compounds to MB5493. Several 9-ene-11α-hydroxy compounds were isolated from *F. sporotrichioides* MB2972 (12). Only tricho-9-ene-2α,3α,11α-triol (compound 8; Fig. 2) was converted to T-2 toxin when fed to cultures of MB5493. The three tetraols (compounds 9 through 11; Fig. 2) were not metabolized. Cyclization of the tetraols would result in 3,8-dihydroxytrichothecene or 3,16-dihydroxytrichothecene; these compounds were not detected in the fed cultures.

Acid interconversions. The YEPD-5G medium used in these experiments had a pH of 6.2 prior to inoculation. After 7 days of growth, the pH of the culture filtrate had dropped to 4.5. Fresh medium adjusted to pH 7.3 caused no conversion of tricho-9-ene-2α,3α,11α-triol (compound 8; see Table 2 and Fig. 2). In acidic medium (pH 6.5 or 5.7), tricho-9-ene-2α,3α,11α-triol was converted to tricho-10-ene-2α,3α,9α-triol (compound 13; Fig. 3). Medium adjusted to a more acidic pH caused both the conversion of tricho-9-ene-2α,3α,11α-triol to tricho-10-ene-2α,3α,9α-triol and the cyclization to 3α-hydroxytrichothecene (compound 2; Fig. 1). The pH of the culture filtrate at 7 days (pH 4.5) is sufficiently acidic to cause a nonenzymatic conversion of tricho-9-ene-2α,3α,11α-triol to 3α-hydroxytrichothecene.

TABLE 2. Nonenzymatic cyclization of tricho-9-ene2α, 3α,-11α-triol[a]

pH	% Cyclization with:		
	11-OH triol	9-OH triol[b]	3-OH[c]
7.3	100.0		
6.5	88.9	11.1	
5.7	82.3	17.3	
5.2	62.3	17.7	20.0
4.7	24.5	20.4	55.1
4.1			100.0

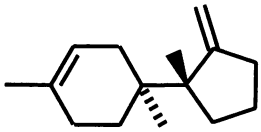
[a] YEPD-5G medium was adjusted to pH 7.3, 6.5, 5.7, 5.2, 4.7, or 4.1 with dilute NaOH or HCl. Tricho-9-ene-2α,3α,-11α-triol (11-OH triol) in dimethyl sulfoxide solution was added to each pH treatment, and the medium was incubated for 7 days at 28°C. Samples were extracted with ethyl acetate, derivatized with TBT, dissolved in hexane, and analyzed by GC.
[b] 9-OH triol, tricho-10-ene-2α,3α,9α-triol.
[c] 3-OH, 3α-hydroxytrichothecene.

DISCUSSION

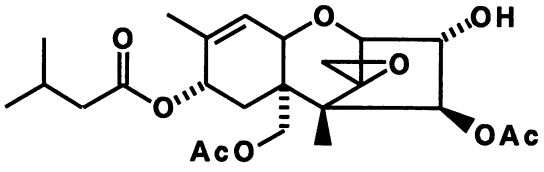
The results of feeding studies with *F. sporotrichioides* MB5493 reported here suggest that there is an ordered sequence of oxidation of the trichothecene skeleton, with oxidation at C-3 preceding that at C-4 or C-15. The isolation of calonectrin analogs (oxygenation at C-3 and C-15) from mutant strain MB2972 (17) and diacetoxyscirpenol (oxygenation at C-3, C-4, and C-15) from mutant strain MB1716 (3) suggests that the order of oxygenation is C-3 → C-15 → C-4 → C-8 in the biosynthesis of T-2 toxin.

Early biosynthetic studies were carried out with *T. roseum*, which accumulates the mycotoxin trichothecin. Nozoe and Machida (12, 16) isolated several trichothecene-type compounds which appeared to be of biogenetic significance. Bioconversion studies with these compounds indicated that trichodiene (compound 15; Fig. 5); trichodiol (compound 12; Fig. 3); and its cyclization product, trichothecene (compound 1; Fig. 1), were intermediates in the biosynthetic pathway of trichothecin (12). These steps have been included in general biosynthetic schemes for other trichothecenes (4), with introduction of hydroxyl, carbonyl, or ester groups occurring after the second cyclization step to trichothecene.

The recent isolation of trichotriol (tricho-10-ene-2α,3α,9α-triol; compound 13; Fig. 3) from *F. sporotrichioides* MC-72083 and the report of its cyclization to 3α-hydroxytrichothecene (compound 2; Fig. 1) in $CDCl_3$ with a trace of water (5) have led to speculation that additional oxygenation may occur prior to the second cyclization in the T-2 toxin pathway (5, 7). The feeding studies reported in the present study support oxidation at C-3 prior to cyclization. Both tricho-9-ene-2α,-3α,11α-triol (compound 8; Fig. 2) and tricho-10-ene-2α,-3α,9α-triol (compound 13; Fig. 3) were converted by MB5493 to T-2 toxin. Our results from incubating



15 Trichodiene



16 T-2 Toxin

FIG. 5. Structures of trichodiene (compound 15) and T-2 toxin (compound 16).

tricho-9-ene-2α,3α,-11α-triol (compound 8) in pH-adjusted media suggest that the second cyclization involves two steps which appear to be nonenzymatic. The bioconversion of tricho-9-ene-2α,3α,11α-triol (compound 8) to T-2 toxin also supports the possibility that 11α-hydroxy compounds play a key role in the biosynthesis of trichothecenes as well as sambucoins and apotrichothecenes.

Trichodiol and trichothecene, biosynthetic precursors of trichothecin in *T. roseum*, were not biotransformed to T-2 toxin by *F. sporotrichioides* MB5493. These results suggest that the two compounds are not biosynthetic precursors of T-2 toxin; however, another possible explanation is that the step blocked in MB5493 is an oxygenation at C-3. If this is the case, the accumulation of no oxygenated trichothecenes in liquid cultures of MB5493 suggests that oxygenation at C-3 is the initial oxygenation in the T-2 toxin biosynthetic pathway. The recent identification of the trichodiene synthase gene (9) may allow for the development of new blocked mutant strains, through gene disruption techniques, that will be suitable for additional feeding experiments to determine the initial oxidation step.

APPENDIX

Tricho-10-ene-2α,3α,9β-triol (compound 14). EI mass spectrum of TMS ether (70 eV), *m/z* (relative intensity): 469 (5), 379 (3), 349 (2), 271 (5), 257 (8), 197 (40), 169 (65), 107 (60), 73 (100). ^{1}H NMR (300 MHz, CD_3OD): δ5.5 (2H, m, H-10, H-11), 4.29 (1H, ddd, J = 4.6 Hz, 7.9 Hz, and 6.3 Hz, H-3), 3.72 (1H, d, J = 4.6 Hz, H-2), 328 (1H, d, J = 4.3 Hz, H-13a), 3.04 (1H, d, J = 4.3 Hz, H-13b), 2.05 (1H, dd, J = 7.8 Hz and 13.4 Hz, H-4a), 1.73-1.40 (5H, m, H-4b, H-7a, H-7b, H-8a, H-8b) 1.27 (3H, d, C-16 Me), 1.06 (3H, s, C-14 Me), 1.01 (3H, s, C-15 Me). ^{13}C NMR (75.5 MHz, CD_3OD): δ77.2 (C-2), 68.6 (C-3), 41.5 (C-4), 43.9 (C-5), 39.7 (C-6), 29.5 (C-7), 34.9 (C-8), 69.0 (C-9), 133.8 (C-10), 133.3 (C-11), 69.2 (C-12), 50.0 (C-13), 20.4 (C-14), 22.0 (C-15), 27.3 (C-16).

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