Target-Oriented Inhibitors of the Late Stages of Trichothecene Biosynthesis. 1. Design, Syntheses, and Proof of Structures of Putative Inhibitors

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Fusarium spp. produce several toxic secondary metabolites (zearalenone, moniliformins, fusarinins, trichothecenes, etc.). Moldy corn toxicosis has been associated with trichothecenes. We have focused in this study on *Fusarium culmorum*, which produces the trichothecenes 3-acetyldeoxynivalenol and sambucinol as major metabolites. We have designed and synthesized putative inhibitors based on the knowledge of the biosynthetic pathway of trichothecenes. A fluorine group was introduced at a strategic position on the trichothecene moiety, or the epoxide at C_{12}–C_{13} was eliminated. The structures of these new compounds were rigorously characterized by spectroscopic techniques.

INTRODUCTION

Trichothecenes are secondary metabolites produced by fungi and in particular by *Fusarium* spp. Due to the widespread nature of *Fusarium* spp., which infect grain crops both in the field and during storage, trichothecenes are of major concern in agriculture. Feed stocks contaminated with trichothecenes are frequently associated with a disease called vomitoxicosis. Deoxynivalenol found in contaminated feed which had caused feed refusal and emesis (vomiting) was called vomitoxin. At present it is unsure if the name was deserved. The acetyl derivative, 3-acetyldeoxynivalenol (3-ADN, Figure 1), is a major metabolite in many *Fusarium* spp. and in particular in *Fusarium culmorum* strain HLX 1503 (Greenhalgh et al., 1984). There is no method at present that can eliminate the production of these mycotoxins efficiently.

In this paper, our approach in the design of inhibitors was based on the knowledge of the biosynthetic pathway of trichothecenes (Zamir and Devor, 1987; Zamir, 1989; Zamir et al., 1987, 1989, 1990). We synthesized modified precursors by introducing a fluorine at different strategic positions. The position at C-15 was chosen since it is the first hydroxylation site after isotrichodermin (Zamir et al., 1991). In addition, since the epoxy function seems to be essential for the toxicity, deepoxy compounds were also synthesized. The new derivatized precursors were characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, mass spectrometry, infrared spectra).

MATERIALS AND METHODS

Proton NMR spectra were measured at 200 or 300 MHz on a Varian XL-300 spectrometer at ambient temperature (22 °C). The samples (2–5 mg) were dissolved in $CDCl_3$. Chemical shifts are reported in δ units using the 7.262 ppm resonance of residual chloroform as internal reference. The carbon-13 and fluorine-19 NMR spectra were obtained on a Varian XL-300 spectrometer operating at 75.43 MHz for carbon and at 282.20 MHz for fluorine. The chemical shifts for carbon-13 are reported in δ units using the central signal of the deuterated chloroform as an internal reference (77.0 ppm). The fluorine chemical shifts are also reported in δ units using a sample of $CFCl_3$ dissolved in chloroform as an external reference. Low-resolution mass spectra were measured on a Hewlett-Packard 5984A quadrupole by direct inlet under ammonia chemical ionization conditions (indicated ammonia pressure 2×10^{-4} Torr, source temperature 210 °C). High-resolution ammonia chemical ionization data on the M + H^+ pseudomolecular ions were collected on a VG Instruments ZAB 2F mass spectrometer under analogous conditions at a resolving power of 10 000 with perfluorokerosene as mass reference. Melting points were obtained on a Büchi 510 melting point apparatus. Analytical thin-layer chromatography was performed using E. Merck glass supported silica gel 60 (F254, 0.25 mm) plates. The compounds were visualized using a ceric sulfate/ammonium molybdate solution [for 1 L of developing agent: 100 mL of H_2SO_4 concentrated, 900 mL of water, 25 g of ammonium molybdate, 10 g of $Ce(SO_4)_2$ (ceric sulfate)]. Column chromatography on silica was accomplished according to the flash chromatography method of Still (Still et al., 1978) (E. Merck silica gel 60, 230–400 mesh). All reactions were conducted under a dry argon atmosphere in flame- or oven-dried glassware. The

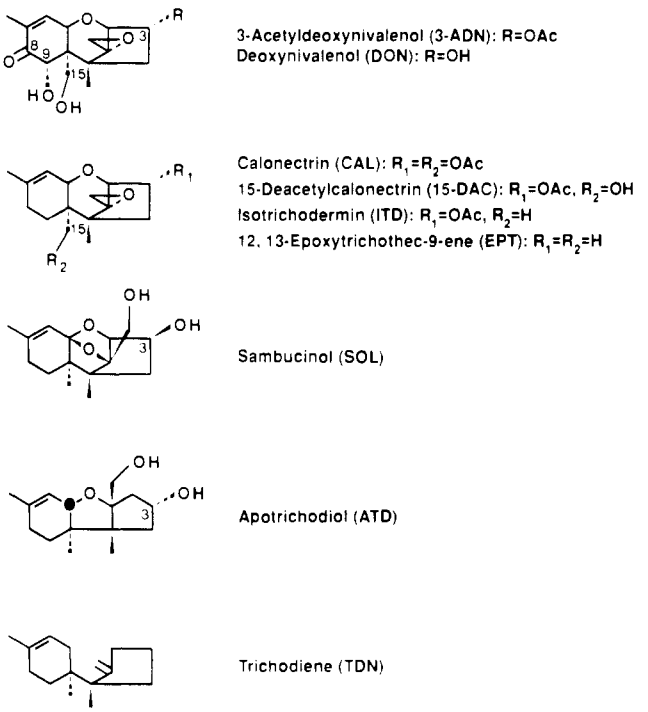

Figure 1. Some metabolites isolated from *F. culmorum*.

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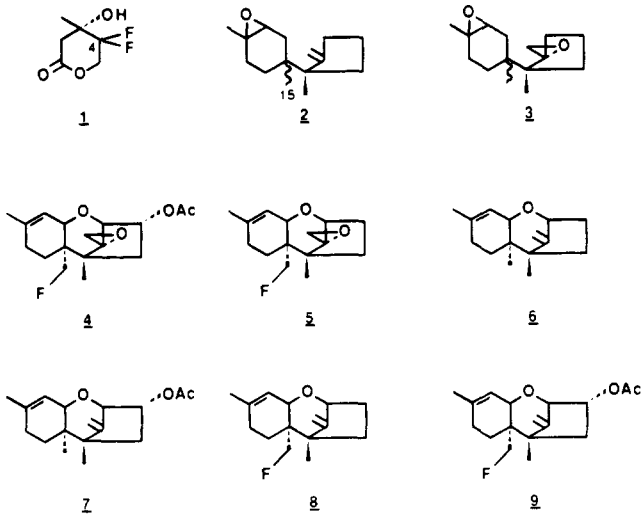

Figure 2. Inhibitors of trichothecene biosynthesis.

following solvents were purified before use: CH_2Cl_2 and dimethylformamide were distilled from CaH_2, toluene was distilled over P_2O_5, methanol (MeOH) was distilled over Mg metal, and tetrahydrofuran (THF) was distilled over lithium aluminum hydride.

Syntheses of Inhibitors to Trichothecene Biosynthesis (Figure 2). 4,4-Difluoromevalonate (1) was synthesized according to the method of Abeles (Reardon and Abeles, 1987).

Epoxidation of trichodiene (2 and 3, Figure 2). The starting material was synthetic trichodiene (Gilbert and Kelly, 1986; Van Middlesworth, 1986) which contained 40% of the stereoisomer at C-15 (bazzanene).

A solution of trichodiene (0.036 g, 0.18 mmol), in 2.6 mL of benzene, was treated with $Mo(CO)_6$ (0.017 g, 0.064 mmol) and subsequently with *tert*-butylhydroperoxide (3 M solution; 0.200 mL, 0.6 mmol). The solution was heated at 65 °C for 1 h, and two new products appeared as observed by TLC. The benzene solvent was evaporated in vacuo. The residue was flash chromatographed (1.6 cm × 7 cm) using 5% ethyl acetate/hexane. Two compounds were isolated as syrups and characterized as 9,10-epoxytrichodiene (**2**, Figure 2) (0.016 g; 41% yield), R_f (10% ethyl acetate/hexane) 0.39, and 9,10,12,13-diepoxytrichodiene (**3**, Figure 2) (0.015 g; 39% yield), R_f (10% ethyl acetate/hexane) 0.17.

^{1}H NMR of **2** (mixture of two isomers in 60:40 ratio indicated as major and minor) (300 MHz, $CDCl_3$): δ 4.893 [m, 1 major, (CH_2)-13A], 4.883 [m, 1 minor, (CH_2)-13A], 4.716 [d, 1 major, J = 2.6 Hz, (CH_2)-13B], 4.631 [d, 1 minor, J = 2.4 Hz, (CH_2)-13B], 2.912 [br s, 1 minor, (CH)-10], 2.863 [dd, 1 major, J = 5.4 Hz, J = 3.1 Hz, (CH)-10], 1.242 [s, 3 major and minor, (CH_3)-16], 0.939 [s, 3 major, (CH_3)-14], 0.914 [s, 3 minor, (CH_3)-14], 0.793 [s, 3 major and minor, (CH_3)-15].

The M + H$^+$ of compound **2** under high resolution was found to have an exact mass of 221.19059 (calculated for $C_{15}H_{24}O$ + H$^+$: 221.19054).

^{1}H NMR of **3** (mixture of two isomers, with no double bonds, in 60:40 ratio) (300 MHz, $CDCl_3$): δ 2.922 [m, 1, (CH)-10], 2.862 [t, 1, J = 4.6 Hz, (CH)-10], 3.288 [d, 1, J = 4.1 Hz, (CH_2)-13A], 3.230 [d, 1, J = 4.2 Hz, (CH_2)-13A], 2.672 [d, 1, J = 4.1 Hz, (CH_2)-13B], 2.708 [d, 1, J = 4.2 Hz, (CH_2)-13B], 1.246 [s, 6, (CH_3)-16], 0.851 [s, 3, (CH_3)-14 or -15], 0.829 [s, 3, (CH_3)-14 or -15], 0.805 [s, 3, (CH_3)-14 or -15], 0.759 [s, 3, (CH_3)-14 or -15].

The M + H$^+$ under high resolution was found to have an exact mass of 237.18551 (calculated for $C_{15}H_{24}O_2$ + H$^+$: 237.18546).

3-Deacetyl-3-O-THP-15-fluoroisotrichodermin (12, Figure 3). 3,15-Deacetyl-3-O-THP-calonectrin (0.050 g, 0.14 mmol) (Zamir et al., 1990), dissolved in 10 mL of dry CH_2Cl_2 under argon at room temperature, was treated with pyridine (0.060 mL, 0.74 mmol). The solution was cooled to 5–10 °C and was treated with triflic anhydride (0.036 mL, 0.21 mmol), dropwise. The orange-pink color of the triflate appeared after 10 min of stirring at 0 °C. The reaction was complete after 30 min as observed by TLC. The solution was diluted with chloroform and washed once with a saturated sodium bicarbonate solution. The organic phase was dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude triflate was used as such for the subsequent reaction.

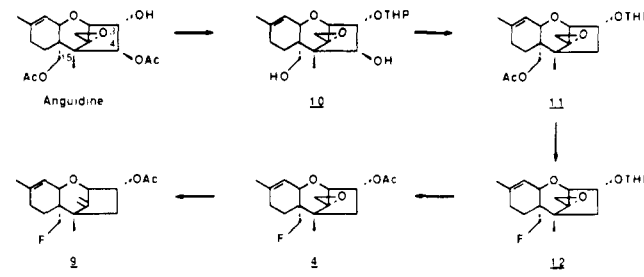

Figure 3. Syntheses of 15-fluoroisotrichodermin (**4**) and 15-fluoro-12,13-deepoxyisotrichodermin (**9**).

To a solution of tetrabutylammonium fluoride (in 0.71 mL, 1 M THF), under argon at room temperature, the above triflate, dissolved in 3 mL of THF, was added. After 15 min of reflux, the reaction was complete. The reaction mixture was diluted in ether and washed with 50% NaCl solution. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The orange needles obtained were flash chromatographed (1.6 cm × 7 cm) using 25% ethyl acetate/hexane. This gave 0.050 g (100% yield) of a clear oil which crystallized at 4 °C, R_f (25% ethyl acetate/hexane) 0.21.

^{1}H NMR of **12** (mixture of two isomers) (300 MHz, $CDCl_3$): δ 5.480 (dq, 1, J = 5.5 Hz, J = 1.5 Hz, CH-10), 5.431 (dq, 1, J = 5.4 Hz, J = 1.5 Hz, CH-10), 4.455 (dt, 1, J = 4.6 Hz, J = 10.7 Hz, CH-3), 4.40 (br dd, 2, $J_{H,F}$ = 47.9 Hz, J = 10.2 Hz, CH_2F-15A), 4.222 (dd, 1, $J_{H,F}$ = 47.1 Hz, J = 10.2 Hz, CH_2F-15B), 4.196 (dd, 1, $J_{H,F}$ = 46.9 Hz, J = 10.2 Hz, CH_2F-15B), 4.249 (dt, 1, J = 4.7 Hz, J = 11.0 Hz, CH-3), 4.130 (o, m, 2, CH-11), 3.626 (d, 1, J = 4.6 Hz, CH-2), 3.619 (d, 1, J = 4.5 Hz, CH-2), 3.043 (d, 1, J = 4.0 Hz, CH-13A), 3.032 (d, 1, J = 3.9 Hz, CH-13A), 2.834 (d, 1, J = 4.0 Hz, CH-13B), 2.830 (d, 1, J = 4.0 Hz, CH-13B), 1.721 (br s, 6, CH_3-16), 0.859 (2 × d, 6, $J_{H,F}$ = 1.4 Hz, CH_3-14).

^{19}F NMR (282 MHz, $CDCl_3$) (ref $CFCl_3$): δ 226.66 (t, 1, CH_2F-15), 226.79 (t, 1, CH_2F-15).

3-Deacetyl-15-fluoroisotrichodermin. A solution of 3-deacetyl-3-O-THP-15-fluoroisotrichodermin (0.50 g, 0.14 mmol) dissolved in 3 mL of dry MeOH, under nitrogen, was treated with pyridinium *p*-toluenesulfonate (0.0040 g, 0.016 mmol). The solution was heated at 60–65 °C for 4.5 h, after which time the reaction was complete. The methanol was evaporated, and the residue was flash chromatographed (1.6 cm × 7 cm) using 50% ethyl acetate/hexane. This gave 0.035 g (92% yield) of purified crystals.

^{1}H NMR of 3-deacetyl-15-fluoroisotrichodermin (300 MHz, $CDCl_3$): δ 5.484 (dq, 1, J = 5.4 Hz, J = 1.5 Hz, CH-10), 4.441 (dt, 1, J = 10.7 Hz, J = 4.2 Hz, CH-3), 4.421 (dd, 1, $J_{H,F}$ = 47.7 Hz, J = 10.2 Hz, CH_2F-15A), 4.195 (dd, 1, $J_{H,F}$ = 47.1 Hz, J = 10.2 Hz, CH_2F-15B), 4.049 (br d, 1, J = 5.4 Hz, CH-11), 3.489 (d, 1, J = 4.6 Hz, CH-2), 3.056 (d, 1, J = 4.1 Hz, CH_2-13A), 2.895 (d, 1, J = 4.1 Hz, CH_2-13B), 2.169 (dd, 1, J = 14.5 Hz, J = 10.7 Hz, CH_2-4A), 1.991 (dd, 1, J = 14.5 Hz, J = 4.2 Hz, CH_2-4B), 1.8–2.1 (m, 4, CH_2-7 and -8), 1.735 (br s, 3, CH_3-16), 0.865 (d, 3, $J_{H,F}$ = 3.3 Hz, CH_3-14).

^{19}F NMR (282 MHz, $CDCl_3$) (ref $CFCl_3$): δ 226.88 (t, 1, $J_{H,F}$ = 47.7 Hz, CH_2F-15).

15-Fluoroisotrichodermin (4, Figure 3). A solution of 3-deacetyl-15-fluoroisotrichodermin (0.032 g, 0.12 mmol) dissolved in 1 mL of acetic anhydride was treated with pyridine (0.1 mL, 1.2 mmol) at room temperature. After 3 h of stirring, the acetylation was complete as monitored by TLC. The mixture was co-evaporated with heptane to dryness. The residue was flash chromatographed (1.6 cm × 7 cm) using 30% ethyl acetate in hexane. This gave 0.037 g (100%) of the desired product as white crystals, R_f (25% ethyl acetate/hexane) 0.21. The ^{1}H NMR is described in Table II. The M + H$^+$ under high resolution was found to have an exact mass of 311.16595 (calculated for $C_{17}H_{23}O_4F$ + H$^+$: 311.16586).

15-Fluoro-12,13-deepoxyisotrichodermin (9, Figure 3). WCl_6 (0.14 g, 0.35 mmol) (Sharpless et al., 1972) was added to 1 mL of dry THF at −78 °C. A dark brown suspension was obtained. *n*-Butyllithium (0.65 mL; 1.6 M solution in hexanes; 1.04 mmol) was added to the above suspension at −78 °C. The mixture was stirred for 15 min at −78 °C and then was allowed to warm to

Table I. ^{1}H NMR Data for Compounds 2 and 3

position	multiplicity	δ (J) 2	δ (J) 3
H_{10} min	(br)	2.912	2.922
H_{10} maj	dd	2.863 (5.4, 3.1)	2.862 (4.6, 4.6)
H_{13A} min	d (o)	4.883	3.288 (4.1)
H_{13A} maj	d (o)	4.893	3.230 (4.2)
H_{13B} min	d	4.631 (2.4)	2.672 (4.1)
H_{13B} maj	d	4.716 (2.6)	2.708 (4.2)
H_{14}/H_{15} maj	2s	0.939, 0.793	0.851, 0.759
H_{14}/H_{13} min	2s	0.914, 0.793	0.829, 0.805
H_{16}	s	1.242	1.246
others	m	2.3–1.1	2.6–1.2

Table II. Characteristic Features of the ^{1}H and ^{19}F NMR of Isotrichodermin Derivatives

position		δ (J) 4	δ (J) 7	δ (J) 9
H_2	d	3.691 (4.6)	4.284 (4.5)	4.313 (4.4)
H_3	dt	5.111 (11.2, 4.6)	4.877 (11.2, 4.6)	4.869 (11.1, 4.4)
H_{10}	dq (br)	5.421 (5.4, 1.5)	5.392 (5.4, 1.6)	5.433 (5.4, 1.4)
H_{11}	d (br)	3.917 (5.2)	3.941 (5.5)	3.967 (5.4)
H_{13A}	d	3.031 (4.0)	5.019 (0.6)	5.071 (s)
H_{13B}	d	2.809 (4.0)	4.703 (0.6)	4.765 (s)
H_{14}	d	0.832 (3.2)	0.984 (s)	1.136 (2.7)
H_{15A}	dd	4.361 (10.1, 47.6)	0.776 (s)	4.432 (10.0, 47.9)
H_{15B}	dd	4.183 (10.1, 47.1)		4.224 (10.0, 47.1)
H_{16}	s (br)	1.682	1.657	1.693
OAc	s	2.053	2.099	2.097
F_{15}	t	−227.2 (47.5)		−227.0 (47.6)

room temperature. 15-Fluoroisotrichodermin (0.027 g, 0.087 mmol), dissolved in 0.5 mL of dry THF, was added dropwise to the mixture at room temperaure. The solution was then heated to 55 °C for 60 min. The mixture was evaporated in vacuo to obtain a black residue which was chromatographed (1.6 cm × 7 cm) using 30% ethyl acetate in hexane. This gave 0.022 g (86% yield) of the required compound as crystals, R_f (25% ethyl acetate/hexane) 0.38. The ^{1}H NMR is described in Table II. The M + H$^+$ under high resolution was found to have an exact mass of 295.17101 (calculated for $C_{17}H_{23}O_3F$: 295.17095).

12,13-Deepoxyisotrichodermin (7, Figure 2). WCl_6 (0.23 g, 0.58 mmol) was added to 1.6 mL of dry THF at −78 °C. A dark brown suspension was obtained. *n*-Butyllithium (1.1 mL; 1.6 M solution in hexanes; 1.76 mmol) was added to the above suspension at −78 °C. The mixture was stirred for 15 min at −78 °C and then was allowed to warm to room temperature. Isotrichodermin (0.035 g, 0.12 mmol), dissolved in 0.6 mL of dry THF, was added dropwise to the mixture at room temperature. The solution was then heated to 50 °C for 40 min. The mixture was evaporated in vacuo to obtain a black residue which was chromatographed (1.6 cm × 7 cm) using 15% ethyl acetate/hexane. This gave 0.022 g (66% yield) of the desired compound as white crystals, R_f (25% ethyl acetate/hexane) 0.42.

^{1}H NMR of **7** (300 MHz, $CDCl_3$): δ 5.192 (dq, 1, J = 5.4 Hz, J = 1.6 Hz, CH-10), 5.019 (br s, 1, CH_2-13A), 4.703 (br d, 1, J = 0.6 Hz, CH_2-13B), 4.885 (d, 1, J = 4.5 Hz, CH-2), 4.877 (dt, 1, J = 11.2 Hz, J = 4.5 Hz, CH-3), 3.941 (br d, 1, J = 5.4 Hz, CH-11), 2.099 (s, 3, OAc), 2.099 (dd, 1, J = 14.5 Hz, J = 4.6 Hz, CH_2-4A), 1.892 (dd, 1, J = 14.5 Hz, J = 11.2 Hz, CH_2-4B), 1.7–1.95 (m, 4, CH_2-7 and -8), 1.657 (br s, 3, CH_3-16), 0.984 (s, 3, CH_3-14 or 15), 0.776 (s, 3, CH_3-15 or -14).

The M + H$^+$ under high resolution was found to have an exact mass of 277.18035 (calculated for $C_{17}H_{24}O_3$ + H$^+$: 277.18037).

15-Fluoro-12,13-epoxytrichothecene (5, Figure 2). A solution of 4-deoxyverrucarol (0.063 g, 0.25 mmol) (Zamir et al., 1990) dissolved in 18 mL of dry CH_2Cl_2, treated with pyridine kept over molecular sieves of 8–12 mesh (0.10 mL, 1.2 mmol), was cooled to 0 °C. Trifluoromethanesulfonic anhydride (0.068 mL, 0.40 mmol) was added dropwise to the solution. The triflate was formed after 30 min at 0 °C as observed by TLC and by the production of the characteristic pinkish brown color of the triflate. The mixture was heated to 40 °C, and 1.26 mL of a 1 M solution of tetrabutylammonium fluoride in THF was added via a syringe in one portion. The reaction was instantaneous as observed by TLC and by the formation of a red color. The mixture was diluted in dichloromethane and was washed with a saturated solution of $NaHCO_3$ (1×) and brine (2×) to neutrality. The organic solution was dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed (1.6 cm × 7 cm) using 20% ethyl acetate/hexane. This gave 0.059 g of the desired compound as crystals (93% yield), R_f (25% ethyl acetate/hexane) 0.32.

^{1}H NMR of **5** (300 MHz, $CDCl_3$): δ 5.366 (dq, 1, J = 5.5 Hz, J = 1.4 Hz, CH-10), 4.385 (dd, 1, $J_{H\text{-}F}$ = 47.9 Hz, J = 10.0 Hz, CH_2F-15A), 4.170 (dd, 1, $J_{H,F}$ = 47.1 Hz, J = 10.0 Hz, CH_2F-15B), 3.648 (d, 1, J = 4.9 Hz, CH-2), 3.597 (br d, 1, J = 5.5 Hz, CH-11), 3.091 (d, 1, J = 4.15 Hz, CH_2-13A), 2.832 (d, 1, J = 4.15 Hz, CH_2-13B), 1.5–2.1 (m, 8, CH_2-3, -4, -7 and -8), 1.669 (br s, 3, CH_3-16), 0.829 (d, 3, $J_{H,F}$ = 3.3 Hz, CH_3-14).

^{19}F NMR (282 MHz, $CDCl_3$) (ref $CFCl_3$): δ 226.73 (t, 1, $J_{H,F}$ = 47.0 Hz, CH_2F-15).

The M + H$^+$ under high resolution was found to have an exact mass of 253.16041 (calculated for $C_{15}H_{21}O_2F$ + H$^+$: 253.16038).

15-Fluoro-9,10,12,13-trichothecadiene (8, Figure 2). WCl_6 (0.18 g, 0.45 mmol) was added to 1.2 mL of dry THF at −78 °C. A dark brown suspension was obtained. *n*-Butyllithium (0.86 mL; 1.6 M in hexanes; 1.38 mmol) was added to the above suspension at −78 °C. The mixture was stirred for 15 min at −78 °C and was then allowed to warm to room temperature. 15-Fluoro-12,13-epoxytrichothecene (0.029 g, 0.11 mmol), dissolved in 0.5 mL of dry THF, was added dropwise to the mixture at room temperature. The solution was then heated to 55 °C for 60 min. The mixture was evaporated in vacuo to obtain a black residue which was chromatographed (1.6 cm × 7 cm) using 30% ethyl acetate/hexane. This gave 0.024 g (88%) of the required compound as a yellow oil, R_f (25% ethyl acetate/hexane) 0.54. The ^{1}H, ^{19}F, and ^{13}C NMR are described in Tables III and IV. The M + H$^+$ under high resolution was found to have an exact mass of 237.16541 (calculated for $C_{15}H_{21}OF$ + H$^+$: 237.16547).

9,10,12,13-Trichothecadiene (6). WCl_6 (0.200 g, 0.504 mmol) was added to 1.4 mL of dry THF at −78 °C. A dark brown suspension was obtained. *n*-Butyllithium (0.96 mL; 1.6 M solution in hexanes; 1.54 mmol) was added to the above suspension at −78 °C. The mixture was stirred for 15 min at −78 °C and was then allowed to warm up to room temperature. 12,13-Epoxytrichothec-9-ene (0.031 g, 0.132 mmol) dissolved in 0.5 mL of dry THF was added dropwise to the mixture at room temperature. The solution was then heated to 50 °C for 30 min. The mixture was evaporated in vacuo to obtain a black residue which was chromatographed (1.6 cm × 7 cm) using 5% ethyl acetate/hexane. This gave 0.024 g (83% yield) of the required compound as a yellow oil, R_f (25% ethyl acetate/hexane) 0.59.

The ^{1}H, ^{19}F, and ^{13}C NMR are described in Tables III and IV. The M + H$^+$ under high resolution was found to have an exact mass of 219.17483 (calculated for $C_{15}H_{22}O$ + H$^+$: 219.17489).

RESULTS AND DISCUSSION

Syntheses of Putative Inhibitors. The rationale for the syntheses of the inhibitors was to introduce a fluorine at a strategic position in the molecules. Fluorine being almost isosteric with hydrogen (van der Waals radii: H, 1.2 Å; F, 1.35 Å) and having a short C–F bond length could therefore lead to substrate mimics which could be irreversible inhibitors. Recent work (Zamir et al., 1991) has shown that the position C-15 is strategic since it seems to be the first hydroxylation site after isotrichodermin. In addition, the epoxide at C_{12}–C_{13} has been shown to be essential for the toxicity of trichothecenes (Joffe, 1983). We therefore also synthesized the deepoxy compounds.

4,4-Difluoromevalonate has been previously synthesized by Abeles and co-workers (Reardon and Abeles, 1987). There are no reported syntheses of the putative inhibitors shown in Figure 2. Compounds **2** and **3** were obtained from epoxidation of synthetic trichodiene (Sharpless and Verhoeven, 1979; Trost et al., 1984; Van Middlesworth, 1986; Gilbert and Kelly, 1986). 15-Fluoroisotrichodermin was prepared according to the scheme shown in Figure 3. Diacetoxyscirpenol isolated from *Fusarium sambucinum* (Richardson and Hamilton, 1987) was the starting material. The first step involves protection of the hy-

Table III. Characteristic Features of the ^1H and ^{19}F NMR of 12,13-Epoxytrichothec-9-ene Derivatives

position		δ (J) 5	6	8
H_2	d	3.720 (4.9)	4.232 (4.9)	4.286 (4.6)
H_{3A}	ddd	1.6–1.9 (m)	1.867 (14.4, 9.5, 5.0)	1.7–1.8 (m)
H_{3B}	ddt		1.688 (14.4, 12.5, 5.0)	1.7–1.8 (m)
H_{10}	dq (br)	5.435 (5.4, 1.7)	5.311 (5.4, 1.5)	5.379 (5.5, 1.4)
H_{11}	d (br)	3.667 (5.4)	3.648 (5.4)	3.603 (5.5)
H_{13A}	d	3.163 (4.1)	4.875 (0.9)	4.959 (0.5)
H_{13B}	d	2.910 (4.1)	4.523 (1.0)	4.621 (0.5)
H_{14}	d	0.899 (3.3)	0.726 (s)	1.127 (3.0)
H_{15A}	dd	4.455 (10.1, 47.9)	0.942 (s)	4.455 (10.0, 47.9)
H_{15B}	dd	4.240 (10.1, 47.1)		4.210 (10.0, 47.2)
H_{16}	s (br)	1.739	1.608	1.680
F_{15}	t	−226.7 (47.8)		−226.4 (48.6)

Table IV. ^{13}C NMR Data for Compounds 6–8

carbon		6	7	8
C_2		79.9	78.2	79.8
C_3		27.4	71.8	32.7 (C_4, C_7 or C_8)
C_4		32.0	39.5	28.3
C_5 or C_6	47.8	48.2		
C_6 or C_5		39.9	40.2	
C_7 or C_8		23.7	24.0	23.3
C_8 or C_7		28.4	28.3	27.2
C_9		139.3	139.1	140.4
C_{10}		119.6	119.6	119.1
C_{11}		70.5	71.8	65.5 (d) ($J_{C,F}$ = 7.8)
C_{12}		155.5	152.2	154.7
C_{13}		102.5	106.3	103.3
C_{14}		16.1	16.2	17.4 (br)
C_{15}		15.9	16.0	83.5 (d) ($J_{C,F}$ = 173.4)
C_{16}		23.3	23.3	19.4 (br)
OAc			170.8, 21.14	

droxyl at C-3 and deacetylation of the acetyls at C-4 and C-15. Reacetylation of **10** gives only an acetyl group at C-15. Elimination of the C-4 hydroxyl group was accomplished according to the Barton deoxygenation procedure (Barton and McCombie, 1975). Deacetylation of **11** yields 3,15-deacetyl-3-*O*-tetrahydropyranylcalonectrin (Jeker et al., 1984). The best method for the fluorination of the neopentyl alcohol at C-15 entails formation of the triflate and its displacement by fluoride. This method was analoguous to the preparation of deoxyhalogeno sugars (Binkley et al., 1980) and the modified version leading to fluorohydrins (Grieco et al., 1979). The triflate at C-15 of 3,15-deacetyl-3-*O*-tetrahydropyranylcalonectrin was very stable (probably stabilized by the 3-*O*-THP ether) and was quantitatively converted to the desired fluoro compound. Deprotection of the C-3 hydroxyl group and acetylation gave compound **4** (15-fluoroisotrichodermin). The steps involved in the synthesis of 15-fluoro-12,13-epoxytrichothec-9-ene (**5**) are shown in Figure 4. The starting material was verrucarol, which was isolated from *Myrothecium verrucaria* (Jarvis et al., 1984). Removal of the 4-hydroxyl was done according to the method of Schuda and co-workers (Schuda et al., 1984). The triflate of 4-deoxyverrucarol could be prepared but was very unstable. It was, therefore, not isolated but was immediately reacted with tetrabutylammonium fluoride solution. The displacement was instantaneous, and the yield of 15-fluoro-12,13-epoxytrichothec-9-ene was 93%. The same tungsten reagent, WCl_6, is used to synthesize the deepoxy compounds (**6–9**) shown in Figure 2, following the procedure of Roush and Russo-Rodriguez (Sharpless et al., 1972; Umbreit and Sharpless, 1981; Colvin and Cameron, 1987; Roush and Russo-Rodriguez, 1987; Cameron and Colvin, 1989).

The NMR spectra of all of the new compounds fit the formulas shown in Figure 2 and are discussed below. High-resolution mass spectra were done for all of the inhibitors

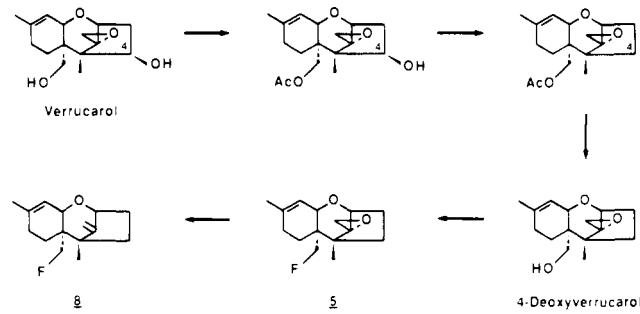

Figure 4. Syntheses of 15-fluoro-12,13-epoxytrichothec-9-ene (**5**) and 15-fluoro-9,10,12,13-trichothecadiene (**8**).

(except for difluoromevalonate, which had been described earlier). The close fit between the observed M + 1 values obtained for the exact masses and the calculated values ensures the validity of the structures shown in Figure 2.

NMR Characterization of the Putative Inhibitors. Comparing the proton NMR spectra of 9,10-epoxytrichodiene (**2**) with that of the parent trichodiene reveals in the deshielded area of the proton spectra the presence of the *exo*-methylene AB protons associated to H_{13} (appearing between 4.9 and 4.6 ppm and coupled with a 2.5-Hz geminal coupling) while the olefinic signal associated to H_{10} is absent from that deshielded area. The H_{10} signal is now observed in a more shielded area (at 2.9 ppm), at a shift characteristic of a proton on an epoxide ring. Also Me_{16}, previously positioned on a double bond (1.7 ppm), is now located at 1.2 ppm, a shift characteristic for a methyl located on a saturated quaternary carbon. These observations are consistent with the presence of a monoepoxy derivative at position 9,10. The two other methyl singlets observed (0.9 and 0.8 ppm) are further split into two peaks having the relative ratio 60:40. These signals have been associated with Me_{14} and Me_{15} existing in the two stereoisomers (bazzanene, trichodiene); the same isomer ratio can also be noted on the H_{13} protons.

The proton NMR spectra of the diepoxy derivative (**3**) reveals the absence of deshielded olefinic protons. The H_{13} AB protons appear as two pairs of doublets (for the two isomers: trichodiene/bazzanene analogue) at about 3.3 and 2.7 ppm and coupled together with a 4.2-Hz coupling. This observation confirms the presence of an epoxy group at position 12,13. The presence of the second epoxy group (at position 9,10) is confirmed by the presence of an H_{10} proton appearing at a shift similar (2.9 ppm) to that observed for the monoepoxy derivative (**2**) and by the observation that Me_{16} is positioned on a saturated carbon (1.2 ppm). The spectra for the diepoxytrichodiene derivative also indicates the presence of two pairs of singlets associated with Me_{14} and Me_{15} existing as two stereoisomers. The most characteristic features of the proton NMR spectra of these two compounds are presented in Table I.

The three isotrichodermin (ITD) derivatives are all characterized by the presence of two deshielded H_2 and H_3 protons coupled together, with the H_3 proton further coupled to two H_4 protons. The coupling constants observed are similar to those reported for the parent ITD compound, confirming that ring C has not been modified. The H_{10}, H_{11}, and Me_{16}, similar in shifts and couplings, confirm the presence of ring A.

Similarly, for the 12,13-epoxytrichothec-9-ene (EPT) derivatives, the presence of a deshielded H_2 proton coupled to one of the shielded H_3 protons, further coupled to shielded H_4 protons, confirms the presence of ring C, identical to the parent EPT compound. The absence of coupling between H_2 and one of the H_3 protons has been interpreted as an indication of a 90° torsion angle, as found in the parent compound. The presence of unmodified ring A is confirmed by the observation of H_{10}, H_{11}, and Me_{16} protons at very similar shifts compared to the parent compound.

In both series of derivatives (ITD and EPT), the modification at position 12,13 from epoxy to deepoxy is monitored nicely by NMR with the disappearance of the epoxy AB system (3.2–2.7 ppm) combined with the appearance of a deshielded AB system characterized by a very small splitting typical of unsaturated geminal protons (0–1.0 Hz). Also, the H_2 proton (of deepoxy-ITD as well as deepoxy-EPT) experienced a significant downfield shift (of about 0.5 ppm) compared with the H_2 of ITD or EPT. With the ITD derivative, we have also observed systematically an upfield shift of about 0.2 ppm with the introduction of the deepoxy group at position 12,13.

The presence of fluorine at position 15 has been confirmed by observing the disappearance of one of the two shielded methyl groups in the proton NMR spectra (0.9–0.7 ppm) combined with the observation of a deshielded AB spin system (4.5–4.2 ppm) further coupled to fluorine with a very large splitting (about 48 Hz). Upon introduction of fluorine at position 15, other minor changes have been observed: the methyl at position 14 is now split into a doublet, as it is coupled with fluorine probably through space (about 3 Hz). The introduction of fluorine is confirmed by the direct observation of a shielded triplet in the ^{19}F NMR spectra.

A summary of the main NMR characteristics of the various derivatives is presented in Table II for ITD, in Table III for the EPT derivatives, and in Table IV for the deepoxy derivatives.

ACKNOWLEDGMENT

We are grateful to the Natural Sciences and Engineering Research Council of Canada for support of this work.

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Received for review June 17, 1991. Revised manuscript received December 12, 1991. Accepted January 7, 1992.