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TWO NEW TRICHOTHECENES PRODUCED BY *FUSARIUM* sp.

KENJI ISHII

Laboratory of Microbial Chemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Shinjuku-ku, Tokyo 162, Japan

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Key Word Index—*Fusarium* sp.; fungi; sesquiterpene; 12,13-epoxytrichothec-9-ene; mycotoxin.

Abstract—Two new 12,13-epoxytrichothecenes have been isolated from the culture filtrate of *Fusarium* sp. strain K-5036 and characterized as 4β,15-diacetoxy-12,13-epoxytrichothec-9-ene-3α,7α-diol and 4β,15-diacetoxy-12,13-epoxytrichothec-9-ene-3α,7α,8α-triol. In addition, the fungus produced diacetoxyscirpenol, nivalenol diacetate and neosolaniol.

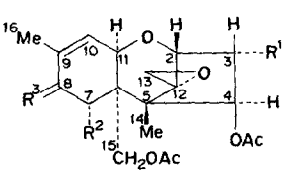
INTRODUCTION

The naturally occurring fungal sesquiterpenoids, having the trichothecene nucleus, have toxic effects on animals, plants and micro-organisms [1]. This class of toxic metabolites was isolated from various species of *Trichothecium*, *Trichoderma*, *Myrothecium*, *Cephalosporium*, *Fusarium* and *Stachybotrys*; and so far 13 trichothecenes have been isolated from species of *Fusarium* [2,3].

Mycotoxicological studies on the metabolites of *Fusarium* spp. led to the discovery of two new trichothecenes. The present paper describes the isolation and structures of the newly discovered toxins.

RESULTS AND DISCUSSION

Toxins were extracted from the culture filtrate of *Fusarium* sp. strain K-5036 grown on Czapek–Dox-peptone medium using charcoal adsorption and methanol elution method [4]. Extraction of methanol–chloroform soluble materials with acetone gave a yellow powder which was divided into 8 fractions (A–H) by chromatography on a Si gel column. Fractions were tested for toxicity using mice and rabbit reticulocytes [5]; toxicity was detected in fractions B–F. TLC analysis of the fractions revealed the presence of diacetoxyscirpenol (**6**) [6,7] and nivalenol diacetate (**7**) [8,9] in fraction B and neosolaniol (**8**) [10,11] in fraction D. Further purifications of fractions C and E by chromatography on Si gel columns gave crystals of (**1**) and (**2**), respectively.



	R^1	R^2	R^3 α	R^3 β
(**1**)	OH	OH	H	H
(**2**)	OH	OH	OH	H
(**3**)	OAc	OAc	H	H
(**4**)	OAc	OH	OAc	H
(**5**)	OAc	OAc	OAc	H
(**6**)	OH	H	H	H
(**7**)	OH	OH	O	
(**8**)	OH	H	OH	H
(**9**)	OH	OH	OAc	H

Compound (**1**) has an empirical formula of $C_{19}H_{26}O_8$ (elemental analysis) and is an isomer of (**8**). The IR spectrum, being similar to that of (**8**), indicated the presence of hydroxyl (3500 cm^{-1}) and acetyl (1720 and 1280 cm^{-1}) groups. The MS (probe) showed the base peak at *m/e* 43 corresponding to acetylation. The M^+ did not appear but M^+-18 (*m/e* 364) and M^+-60 (*m/e* 322) indicating the presence of hydroxyl and acetoxy groups occurred in the high mass region.

The NMR spectrum of (**1**) showed signals characteristic of the naturally occurring trichothecenes such as the *AB* quartet at δ3·07 and 3·17 arising from the spiroepoxy protons (H-13) and the two methyl singlets at δ1·13 and 1·75 due to the tertiary methyl (H-14) and the allylic methyl (H-16) groups, respectively. The presence of two

Table 1. Chemical shifts (δ values) of protons in (**1**) and (**2**) and their acetates

Compound	2-H	3-H	4-H	7-H	8-H	10-H	11-H	13-H	14-H	15-H	16-H	OAc
(1)	3·70*d* (5)	*ca* 4·25	5·20*d* (3·5)	4·62*dd* (6,10)	*ca* 2·0, 2·43*dd*	5·50*d* (6)	*ca* 4·25	3·07*d*, 3·17*d* (4)	1·13*s*	4·24*d*, 4·42*d* (12)	1·75*s*	2·07*s*, 2·15*s*
(2)	3·70*d* (5)	*ca* 4·2	5·45*d* (3)	4·01*d* (5)	*ca* 4·5	5·65*d* (6)	*ca* 4·5	3·05*d*, 3·15*d* (4)	1·10*s*	4·29*d*, 4·50*d*	1·85*s*	2·02*s*, 2·13*s*
(3)	3·88*d*	5·17*d*	6·10*d*	5·73*d*	*ca* 2·05, 2·65*dd*	5·55*d*	4·42*d*	2·64*d*, 3·10*d*	0·90*s*	4·18*d*, 4·65*d*	1·70*s*	2·05*s*, 2·08*s*, 2·11*s*, 2·14*s*
(4)	3·90*d*	5·20*d*	5·92*d*	4·70*d*	5·50*d*	5·80*d*	4·32*d*	3·10*d*, 3·25*d*	1·10*s*	4·35*d*, 4·55*d*	1·78*s*	2·08*s*, 2·11*s*, 2·11*s*, 2·14*s*
(5)	3·90*d*	5·18*d*	6·05*d*	5·60*d*	5·85*d*	5·80*d*	4·55*d*	2·65*d*, 3·05*d*	0·85*s*	4·40*d* 4·70*d*	1·80*s*	2·04*s*, 2·1*s*, 2·14*s*, 2·14*s*, 2·19*s*

Coupling constants in parentheses; *s*, singlet; *d*, doublet; *dd*, double doublet.

acetyl groups were revealed by two 3-proton singlets at δ2·07 and 2·14, and the chemical shifts of the signals due to H-4 (δ5·20) and H-15 (δ4·33) suggested the presence of acetoxy groups at positions 4 and 15. The presence of a hydroxyl group at position 3 was also indicated by the chemical shift of the signal due to H-3 (δ4·25) which is coupled with the signals due to H-2 (δ3·70) and H-4 (δ5·20). The coupling constants, $J_{2,3}$ (5 Hz) and $J_{3,4}$ (3·5 Hz), indicated the orientation of the hydroxyl group at position 3 was α and that of the acetoxy group at position 4 was β.

Spin-decoupling experiments revealed the presence of an *ABX* system at δ2·05, 2·43 and 4·62. The signals were due to the partial structure $-CH_2-CH(OH)-$ which could occupy position 7–8. The coupling constants of the *X* signal at δ4·62 (*J* 6, 10 Hz) indicated a 7α- or 8β-hydroxyl group. On the other hand, deshielding of H-14 (δ1·13) compared with that of (**8**) (δ0·85) suggested the presence of a 7α-oriented hydroxyl group in (**1**) [12].

The structure of (**1**) was confirmed by analysis of the NMR spectrum of its diacetate (**3**). Acetylation resulted in downshifts of the signals due to H-3 (0·92 ppm) and H-7 (1·11 ppm), and an upshift of the signal due to H-14 (0·23 ppm).

Compound (**2**), $C_{19}H_{26}O_9$, showed an IR spectrum similar to those of (**1**) and (**8**). The MS showed the M^+ at *m/e* 398 and the base peak at *m/e* 43. The NMR spectrum indicated the presence of 3α-hydroxyl, 7α-hydroxyl, 4β-acetoxy and 15-acetoxy groups as in the case of (**1**).

The significant difference between the NMR spectra of (**1**) and (**2**) was that the latter did not show the methylene signal due to H-8. This difference and the chemical formula suggested that one hydroxyl group was present at position 8. The orientation of the hydroxyl group on C-8 was revealed by acetylation of (**2**) and measuring the coupling constant $J_{7,8}$. Acetylation gave a diacetate (**4**) and a triacetate (**5**); the coupling constant of the signal due to H-8 (5 Hz) in the NMR spectrum of (**4**) indicated a 8α-hydroxyl group.

Chemical proof of the structure of (**2**) was provided by the $NaBH_4$ reduction of (**7**) to (**2**). The reaction was previously reported [13] although mp of the product obtained in the previous study was different from this compound. A triacetate (**9**) of the parent alcohol of (**2**) was previously isolated from *F. scirpi* [6,14]. *Fusarium* sp. K-5036 thus produces five toxins (**1**, **2**, **5**, **7**, and **8**) which differ only in their substitutions at positions 7 and 8 in the trichothecene nucleus.

EXPERIMENTAL

Mp's are uncorrected. IR spectra were taken as KBr pellets. NMR spectra were measured for solutions in $CDCl_3$ with TMS as internal standard. Kieselgel 60 and Kieselgel G (Merck) were used for column and TLC, respectively. For PLC, 500 μm adsorbent layers were used. Spots on TLC plates were made visible by spraying with 20% H_2SO_4 and heating at 110°.

Culture of fungus. The strain of *Fusarium* sp. K-5036 used in this study was isolated from a sample of river water and kindly donated by Dr. Y. Matsuda, Koube City Institute of Environmental Hygienics. Inoculum was grown at 25° on potato-dextrose agar slant for 14 days. Conidia and mycelia of the fungus were seeded in 100 Fernbach flasks each containing 250 ml of Czapek-Dox-peptone soln [4] and incubated stationary at 25° for 14 days.

Extraction of toxins. Procedure for extraction of toxins from the culture filtrate was carried out with the method previously reported [4] with modifications. Culture filtrate (21·5 l.) was mixed with activiated charcoal (200 g) and kept at room temp. for 3 hr with occasional stirring. The charcoal collected by filtration was washed with H_2O and the materials adsorbed on the charcoal were eluted 2× with MeOH (4, 2 l.). Combined MeOH eluates were evaporated to dryness and redissolved in 250 ml of hot MeOH; after removal of insoluble materials, the soln was concentrated to 100 and 500 ml of $CHCl_3$ was added resulting in formation of ppt. The MeOH–$CHCl_3$ soln was filtered and the filtrate was evaporated to dryness; the powdered material (10·5 g) was extracted with 300 ml of Me_2CO under reflux to yield Me_2CO soluble material (5·5 g).

4β,15-diacetoxy-12,13-epoxytrichothec-9-ene-3α,7α-diol. Me_2CO extract was chromatographed on a column (4 × 80 cm) packed with Si gel and developed with C_6H_6–Me_2CO (5:2, 1500 ml; 2:1, 1500 ml; 1:1, 1000 ml) followed by Me_2CO (1000 ml) and MeOH (1000 ml). The eluate was monitored by TLC and divided into 8 fractions (A 99, B 434, C 288, D 94, E 532, F 707, G 445, H 2000 mg) according to the R_f values of the components. Fraction C (280 mg) was further purified by chromatography on a Si gel column (1·5 × 40 cm) with *n*-hexane–Me_2CO (1:1). The purified material which gave R_f 0·5 on TLC with the same system (200 mg) was crystallized from $CHCl_3$ and *n*-hexane as needles. The compound was recrystallized 2× from the same solvents (107 mg), mp 201–203° (Found: C, 59·25; H, 6·78. $C_{19}H_{26}O_8$ requires: C, 59·65; H, 6·85%); IR bands at 3500, 2950, 1720, 1380, 1280 and 1060 cm^{-1}; MS *m/e* 364, 322, 304 and 263.

4β,15-diacetoxy-12,13-epoxytrichothec-9-ene-3α,7α,8α-triol. Fraction E (525 mg) was also chromatographed on a Si gel column (1·5 × 50 cm) with *n*-hexane–Me_2CO (1:1). The material giving R_f 0·38 on TLC with the same solvent system (380 mg) gave needles from EtOH–Et_2O–*n*-hexane. The compound was recrystallized from the same solvents (200 mg), mp 167–169° (Found: C, 56·56; H, 6·50. $C_{19}H_{26}O_9$ requires C, 57·28; H, 6·58%); IR bands at 3500, 2950, 1750, 1390, 1260 and 1050 cm^{-1}; MS *m/e* 398, 380, 338, 320, 277 and 261.

Acetylation of (**1**). (**1**) (30 mg), pyridine (1·5 ml) and Ac_2O (1·5 ml) was set aside at room temp. for 24 hr. The product was purified by PLC, developed with *n*-hexane–Me_2CO (2:1), and eventually crystallized from C_6H_6–*n*-hexane to give 3α,4β,7α,15-tetraacetoxy-12,13-epoxytrichothec-9-ene as needles (25 mg), mp 147–149°.

Acetylation of (**2**). (**2**) (25 mg) in pyridine (5 ml) and Ac_2O (5 ml) was kept at room temp. for 18 hr. The crude product was subjected to PLC with *n*-hexane–Me_2CO (2:1). Two major bands (R_f 0·36 and 0·27) were scraped off and eluted with Me_2CO. Solid obtained from the lower band was crystallized with C_6H_6–*n*-hexane to give 3α,4β,8α,15-tetraacetoxy-12,13-epoxytrichothec-9-ene-7α-ol as needles (9·5 mg), mp 207·5–209·5°. Evaporation of eluate from the upper band gave amorphous 3α,4β,7α,8α,15-pentaacetoxy-12,13-epoxytrichothec-9-ene (12 mg).

$NaBH_4$ reduction of (**7**). (**7**) (50 mg) in MeOH (2 ml) was treated at 0° with $NaBH_4$ (35 mg) in MeOH (1 ml) and kept at room temp. for 2 hr. Addition of H_2O and HOAc (to pH 5) was followed by evaporation of MeOH and continuous extraction with $CHCl_3$ for 2 hr. The extract was then subjected to PLC with *n*-hexane–Me_2CO (1:1). The band corresponding to (**2**) (R_f 0·24) was scraped off and eluted with Me_2CO. Evaporation of eluate gave a solid which crystallized from EtOH–Et_2O–*n*-hexane as needles. The mp, IR and NMR spectra of the crystal were identical with those of (**2**).

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