N-Acetyl Derivatives of Type C Fumonisins Produced by *Fusarium oxysporum*

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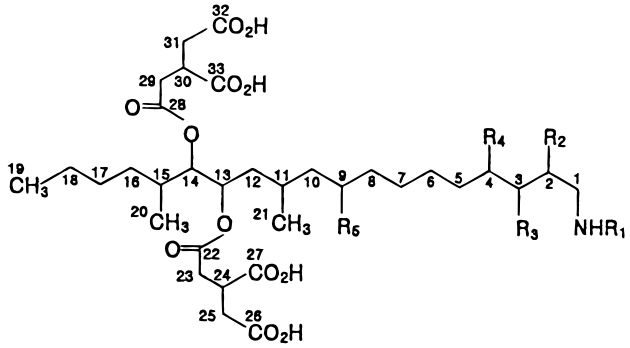
Four new fumonisins, an isomer of fumonisin C_1 (iso-FC_1) (**1**) and three *N*-acetyl derivatives of hydroxylated fumonisin C_1 (OH-FC_1) (**2**), fumonisin C_1 (FC_1) (**3**), and iso-FC_1 (**4**) were isolated from wheat cultures of *Fusarium oxysporum*. One of the hydroxy groups of compound **1** is at C-3 instead of C-4 for FC_1. Compounds **2**−**4** are similar in structure to OH-FC_1, FC_1, and iso-FC_1, respectively, except for the presence of an acetyl group at the amine of C-1.

Fumonisins are a structurally related group of mycotoxins commonly found in corn and corn-based food products.[1] Fumonisins are characterized by a 19- or 20-carbon aminopolyhydroxyalkyl chain, which is diesterified with propane-1,2,3-tricarboxylic acid (TCA). A-, B-, C-, and P-types, as well as some partially hydrolyzed fumonisins, have been isolated and characterized from cultures of *Fusarium* species.[2,3] The A-series of fumonisins are acetylated on the amino group, while the B-series have a free amine. The C-series of fumonisins is structurally similar to the B-series; however, the C-1 terminal methyl group is missing. The chemical structures of fumonisin C_1 (FC_1), fumonisin C_3 (FC_3), and fumonisin C_4 (FC_4) are identical to those of FB_1, fumonisin B_3 (FB_3), and fumonisin B_4 (FB_4), respectively, except that the C-1 terminal methyl group is missing. Hydroxylated FC_1 (OH-FC_1) has an additional hydroxy group at C-3.[4] In the course of screening for fumonisins produced in cultures of *Fusarium* species, one of the *F. oxysporum* isolates was found to produce unknown fumonisin analogues when its culture extract was analyzed by electrospray LCMS. This report describes the isolation and characterization of an isomer of FC_1 (iso-FC_1, **1**) and three *N*-acetyl derivatives of each; OH-FC_1 (**2**), FC_1 (**3**), and iso-FC_1 (**4**) produced by *F. oxysporum* KCTC 16654 (Tuberculariaceae).

Compounds **1**−**4** were isolated from wheat cultures of *F. oxysporum* KCTC 16654 and characterized by FABMS, electrospray LCMS, and NMR spectrometry. The molecular weight of each of the four toxins was determined by FABMS and LCMS: 708 ($[M + H]^+$) for **1**, 766 ($[M + H]^+$) for **2**, 750 ($[M + H]^+$) for **3**, and 750 ($[M + H]^+$) for **4**. The protonated molecular ion of **1** was consistent with that of FC_1, indicating that **1** is an isomer of FC_1. FC_1 and **1** were similarly resolved on TLC using various solvent systems, but had different color reactions to 0.5% *p*-anisaldehyde after charring; FC_1 showed purple on TLC, while **1** appeared violet. The protonated molecular ions at *m*/*z* 766 (**2**), 750 (**3**), and 750 (**4**) suggested that **2** was an *N*-acetyl derivative of OH-FC_1 and that **3** and **4** were *N*-acetyl derivatives of FC_1 or **1**. In addition, FABMS of compounds **1**−**4** exhibited two abundant fragment ions corresponding to $[M + H - 176]^+$ and $[M + H - 352]^+$, which are indicative of successive losses of two TCA groups.



	R_1	R_2	R_3	R_4	R_5
1	H	OH	OH	H	OH
2	$COCH_3$	OH	OH	OH	OH
3	$COCH_3$	OH	H	OH	OH
4	$COCH_3$	OH	OH	H	OH

The ^{13}C NMR data of **1**−**4** are shown in Table 1. The NMR data of ^1H, ^{13}C, DEPT 135°, $^1H-^1H$ DQF COSY, and $^1H-^{13}C$ COSY experiments of **1** were consistent with those of FC_1 except for the position of one hydroxy group. It was determined that compound **1** contained three hydroxy groups at C-2, C-3, and C-9 instead of at C-2, C-4, and C-9 in FC_1. In the $^1H-^1H$ DQF COSY spectrum of **1**, a downfield proton (H-2, δ 3.61) was correlated to two downfield protons H-1_a5 (δ 2.94) and H-1_b (δ 3.19) and another downfield proton H-3 (δ 3.51), indicating that two of the three hydroxy groups were located at C-2 and C-3. H-3 was correlated to two methylene protons H-4_a (δ 1.36) and H-4_b (δ 1.52). The presence of the other hydroxy group at C-9 of **1** was determined by comparison of the $^1H-^1H$ DQF COSY spectra of **1** and FC_1; in $^1H-^1H$ DQF COSY spectra of both compounds, a downfield proton of H-9 (δ 3.61) coupled with four upfield protons (H-8_a, H-8_b, H-10_a, and H-10_b) of methylene region. Thus, the structure of **1** was elucidated as 1-amino-11,15-dimethyl-2,3,9,13,14-pentahydroxynonadecane, which is esterified at both the 13 and 14 positions with TCA. Comparison of this metabolite to the B series of fumonisins suggests that **1** is similar in structure to iso-fumonisin B_1 except for the terminal methyl group.[6] The ^1H and ^{13}C NMR data of compounds **2**−**4** were identical to those of OH-FC_1,[4] FC_1,[7] and **1**, respectively, except for the presence of an acetyl group ($COCH_3$) at the amine of C-1. The A-series fumonisins, *N*-acetyl amides of the B-series fumonisins, were believed to be byproducts of the extraction procedures because HOAc was commonly

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Table 1. ^{13}C NMR Shifts for **1**−**4** in CD_3OD[a]

carbon	1	2	3	4
1	43.7	44.1	47.1	43.4
2	71.8	71.5	68.4	74.8
3	73.6	75.0	42.8	73.9
4	33.9	69.8	68.9	33.9
5	30.7	36.5	38.9	30.8
6	26.5[b]	26.9[b]	26.8[b]	26.8[b]
7	26.7[b]	26.7[b]	26.7[b]	26.9[b]
8	39.5[c]	39.2[c]	39.3[c]	39.3[c]
9	69.9	71.0	69.8	69.9
10	44.5	44.4	44.5	44.5
11	26.9	26.8	26.7	26.9
12	39.5[c]	39.0[c]	39.0[c]	39.1[c]
13	72.9	73.0	73.0	73.1
14	78.6	78.6	78.7	78.7
15	34.8	34.6	34.8	34.8
16	33.1	33.0	33.0	33.1
17	29.6	29.5	29.6	29.6
18	23.9	23.8	23.8	23.8
19	14.4	14.4	14.4	14.4
20	15.9	15.9	15.9	15.9
21	20.7	20.5	20.6	20.6
22	173.4[d]	173.1[d]	173.3[d]	173.2[d]
23	37.0[e]	36.3[e]	36.7[e]	36.2[e]
24	39.2[f]	38.6[f]	38.9[f]	38.7[f]
25	37.5[g]	36.3[g]	36.7[g]	36.6[g]
26	178.6[h]	177.2[h]	177.7[h]	177.1[h]
27	178.2[h]	176.9[h]	177.5[h]	176.8[h]
28	173.3[d]	172.9[d]	173.1[d]	173.0[d]
29	36.8[e]	36.2[e]	36.7[e]	36.1[e]
30	39.2[f]	38.6[f]	38.9[f]	38.6[f]
31	37.3[g]	36.4[g]	36.6[g]	36.5[g]
32	177.0[i]	175.5[i]	176.0[i]	175.4[i]
33	176.6[i]	175.3[i]	175.8[i]	175.3[i]
34		174.2[j]	175.5[j]	174.1[j]
35		22.6[k]	22.6[k]	22.5[k]

[a] Chemical shifts in ppm (δ) from CD_3OD (49.0 ppm) signal. [b−i] Shift assignments with identical superscripts may be interchanged. [j,k] The chemical shifts indicated carbonyl and methyl carbons of the acetyl group at the amino group of C-1.

used in the isolation procedure for the fumonisins. However, Musser et al.[3] reported that the A-series fumonisins were detected along with the B-series fumonisins in most of the cultures of *Fusarium* species. The *N*-acetyl keto form of fumonisin (FAK_1) was also obtained from solid cultures of *F. proliferatum*.[8] In this study, three *N*-acetyl derivatives of the C series of fumonisins were detected on TLC in the collected fractions from the Amberlite XAD-2 column using only aqueous MeOH as an eluent. In addition, the culture extract of *F. oxysporum* with MeOH−H_2O (3:1) was directly analyzed by LCMS, and the protonated ion signals were detected at *m*/*z* 766 for **2**, 750 for **3**, and 750 for **4**. This indicated that compounds **2**−**4** were originally present in the solid culture of *F. oxysporum* and are not artifacts. This isolate of *F. oxysporum*, KCTC 16654, also produced low levels of the B-series fumonisins, including FB_1, FB_2, and FB_3, as well as high levels of the C-series fumonisins, including OH-FC_1, FC_1, FC_3, and FC_4 , which were previously identified in an isolate of *F. oxysporum* from *Dianthus caryophyllus*.[4]

Experimental Section

General Experimental Procedures. FABMS were collected on a JEOL JMS-AX 505 mass spectrometer with a glycerol matrix and Ar as the bombarding gas. Electrospray LCMS was conducted using a Finnigan LCQ mass spectrometer equipped with Hewlett−Packard HPLC (Palo Alto, CA). ^1H and ^{13}C NMR, $^1H-^1H$ DQF and $^1H-^{13}C$ COSY, and DEPT 135° spectra were recorded in CD_3OD on a JEOL Lambda 400 spectrometer, and spectra were referenced to TMS (^1H) or to solvent signals (^{13}C).

Fungal Materials. The fungal isolate was *F. oxysporum* KCTC 16654, which had been isolated from *Asparagus officinalis* by our laboratory. This isolate was highly pathogenic and caused wilt disease in the asparagus. The isolate was deposited in the Korean Collection for Type Culture (KCTC) at the Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea, and the accession number was attached by the KCTC. Erlenmeyer flasks (1 L), each containing 200 g of wheat and 120 mL of distilled H_2O, were autoclaved for 1 h at 121 °C twice with a 24-h interval. The wheat was inoculated with mycelial plugs from a 5-day-old PDA plate of the fungus. The flasks were incubated for 4 weeks at 25 °C. The mycelial mass and substrate were dispersed onto a screen-bottom tray and allowed to air-dry. This dried substrate was ground into a flour.

Extraction and Isolation. Solid culture material (1.5 kg) was extracted four times with MeOH−H_2O (3:1) (totalling 9 L) and filtered through a Buchner funnel. The filtrate was concentrated to dryness and dissolved in 100 mL of H_2O. The aqueous solution was applied onto an Amberlite XAD-2 column (55 × 600 mm, 1 kg, 20−60 mesh; Sigma Chemical Co., MO). The column was washed with 1 L of H_2O and successively eluted with MeOH−H_2O (1:3, 1 L), MeOH−H_2O (1:1, 1 L), MeOH−H_2O (3:1, 2 L), and MeOH (4 L). Each fraction was analyzed by analytical TLC using $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1) as the developing solvent system. Fumonisins were visualized by spraying the TLC plate with 0.5% *p*-anisaldehyde solution in MeOH−H_2SO_4−HOAc (85:5:10) and heating it at 110 °C for 10 min. Compound **1** was eluted in the MeOH−H_2O (1:3) and MeOH−H_2O (1:1) fractions, and compounds **2**−**4** were eluted in the MeOH−H_2O (3:1) and MeOH fractions. The fractions were monitored by TLC and reduced to two fractions called F1 and F2.

The F1 fraction (ca. 41 g) was dissolved in 90 mL of $CHCl_3$−MeOH−HOAc (6:3:1) and applied onto a Si gel column (50 × 500 mm, 300 g, 70−230 mesh, E. Merck). The column was eluted with $CHCl_3$−MeOH−HOAc (6:3:1) and the eluate was collected in 15-mL aliquots with a fraction collector. The fractions containing **1** were combined and concentrated to dryness. The residue containing **1** (ca. 1.95 g) was dissolved in a minimal volume of $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1) and fractionated on a Si gel column (20 × 600 mm, 90 g, 230−400 mesh, E. Merck) using the same solvent. Fractions were collected in 10-mL aliquots with a fraction collector and analyzed by TLC. The fractions containing **1** were combined, concentrated to dryness, and then further separated with a preparative HPLC (Waters Delta Prep 4000, Millipore Co., MA) through a preparative μBondapak C_{18} column (25 × 100 mm, 10 μm, Millipore Co., MA) using a gradient mobile phase of MeOH−H_2O−HOAc (20:80:1) to MeOH−HOAc (100:1) at a flow rate of 10 mL/min for 50 min. The fractions (20 mL each) containing **1** were combined and concentrated to dryness. Because compound **1** was still contaminated with some pigments, **1** was finally purified with a strong-anion exchange (SAX) cartridge (Sep-Pak Accell Plus QMA, 35 mL, Millipore Co., MA). The residue (300 mg) was dissolved in 35 mL of MeOH−H_2O (3:1) and applied to a SAX cartridge, which was successively washed with MeOH−H_2O (3:1, 35 mL) and MeOH (35 mL) and eluted with 1% HOAc in MeOH (50 mL). Compound **1** came off with 1% HOAc in MeOH. The fractions containing **1** were combined and concentrated to dryness to yield 150 mg.

The F2 fraction containing compounds **2**−**4** (ca. 44 g) was dissolved in 100 mL of $CHCl_3$−MeOH−HOAc (6:3:1) and applied onto a Si gel column (50 × 500 mm, 300 g, 70−230 mesh, E. Merck). The column was eluted with $CHCl_3$−MeOH−HOAc (6:3:1), and the eluate was collected in 15-mL aliquots with a fraction collector. The fractions containing **2**−**4** were combined and then concentrated to dryness. The residue (ca. 27 g) was redissolved in 25 mL of $CHCl_3$−MeOH−H_2O (14:6:1) containing 1% HOAc and fractionated on a Si gel column (25 × 500 mm, 170 g, 230−400 mesh, E. Merck) using the same solvent as the mobile phase. Fractions (10 mL each) were collected, analyzed by TLC as described above, and combined to give three fractions containing **2**, **3**, or **4**. Each fraction was

applied onto a reversed-phase (C_{18}) column (30 × 250 mm, 150 g, 40−63 μm, E. Merck). The columns were successively eluted with MeOH−H_2O (1:4, 500 mL), MeOH−H_2O (2:3, 500 mL), MeOH−H_2O (3:2, 1 L), MeOH−H_2O (4:1, 1 L), and MeOH (1 L). Compounds **2**−**4** were eluted with MeOH−H_2O (3:2) and MeOH−H_2O (4:1). Fractions containing **2**−**4** were concentrated to dryness to give 490 mg of **2**, 230 mg of **3**, and 90 mg of **4**.

Iso-FC$_1$ (1): colorless liquid; R_f = 0.31, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.14 (1H, dt, J = 9.3, 3.2 Hz, H-13), 4.95 (1H, dd, J = 7.3, 3.9 Hz, H-14), 3.61 (2H, H-2, H-9), 3.51 (1H, H-3), 3.19 (1H, dd, J = 13.0, 3.4 Hz, H-1$_b$), 3.14 (2H, m, H-24, H-30), 2.94 (1H, dd, 12.8, 8.8, H-1$_a$), 2.74−2.43 (8H, m, H-23, H-25, H-29, H-31), 1.81 (1H, m, H-11), 1.68 (2H, H-15, H-12$_b$), 1.25−1.60 (17H, m), 1.09 (1H, H-10$_a$), 1.07 (1H, H-16$_a$), 0.94 (6H, Me-20, Me-21), 0.89 (3H, t, J = 6.7 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; positive FABMS m/z 708 [M + H]$^+$ (100), 690 (6), 672 (3), 532 (4), 356 (17), 338 (8), 320 (4).

***N*-Acetylated OH−FC$_1$ (2):** white powder; R_f = 0.45, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.05 (1H, dt, J = 9.3, 3.0 Hz, H-13), 4.87 (1H, dd, J = 7.3, 3.4 Hz, H-14), 3.71 (1H, H-4), 3.64 (1H, td, J = 8.0, 3.4 Hz, H-2), 3.53 (1H, m, H-9), 3.43 (1H, dd, J = 12.9, 3.7 Hz, H-1$_b$), 3.22 (1H, H-1$_a$), 3.15 (1H, dd, J = 8.0, 3.7 Hz, H-3), 3.09 (2H, m, H-24, H-30), 2.74−2.38 (8H, m, H-23, H-25, H-29, H-31), 1.89 (3H, s, $COCH_3$), 1.71 (1H, m, H-11), 1.59 (1H, H-15$_b$), 1.51 (1H, H-12$_b$), 1.21−1.47 (15H, m), 1.08 (1H, H-10$_a$), 1.01 (1H, H-16$_a$), 0.85 (6H, Me-20, H-21), 0.80 (3H, t, J = 5.7 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; positive FABMS m/z 766 [M + H]$^+$ (100), 748 (11), 730 (14), 590 (4), 414 (22), 396 (16), 378 (7).

***N*-Acetylated FC$_1$ (3):** white powder; R_f = 0.52, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.05 (1H, dt, J = 11.3, 3.7 Hz, H-13), 4.88 (1H, dd, J = 7.3, 3.7 Hz, H-14), 3.81 (1H, m, H-2), 3.71 (1H, m, H-4), 3.52 (1H, m, H-9), 3.17 (1H, dd, J = 12.0, 4.3 Hz, H-1$_b$), 3.03 (3H, m, H-1$_a$, H-24, H-30), 2.74−2.39 (8H, H-23, H-25, H-29, H-31), 1.87 (3H, s, $COCH_3$), 1.72 (1H, m, H-11), 1.59 (1H, H-15), 1.51 (1H, H-12$_b$), 1.40−1.08 (17H, m), 1.01 (1H, m, H-10$_a$), 0.98 (1H, H-16$_a$), 0.86 (6H, d, J = 5.4 Hz, Me-20, Me-21), 0.80 (3H, d, J = 6.6 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; positive FABMS m/z 750 [M + H]$^+$ (100), 732 (30), 714 (26), 574 (4), 556 (15), 538 (11), 398 (16), 380 (66), 362 (59).

***N*-Acetylated iso-FC$_1$ (4):** white powder; R_f = 0.50, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.05 (1H, dt, J = 13.2, 4.0 Hz, H-13), 4.88 (1H, dd, J = 8.0, 2.9 Hz, H-14), 3.53 (1H, m, H-9), 3.35 (2H, H-1$_b$, H-2), 3.29 (1H, H-3), 3.15 (1H, H-1$_a$), 3.09 (2H, m, H-24, H-30), 2.74−2.38 (8H, m, H-23, H-25, H-29, H-31), 1.87 (3H, s, $COCH_3$), 1.72 (1H, m, H-11), 1.61 (1H, H-15), 1.15−1.47 (18H, m), 1.01 (1H, H-10$_a$), 0.98 (1H, H-16$_a$), 0.86 (6H, Me-20, Me-21), 0.80 (3H, d, J = 6.8 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; positive FABMS m/z 750 [M + H]$^+$ (100), 732 (28), 714 (7), 574 (5), 556 (3), 398 (16), 380 (29), 362 (14).

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