

# Isolation and Structure Elucidation of Four Fatty Acid Derivatives of the Mycotoxin Fusarochromanone Produced by *Fusarium equiseti*

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Four compounds were found in rice cultures of *Fusarium equiseti* (Alaska 2-2). Analysis by mass spectrometry (EI, PCI, and FAB) indicated that compounds I, II, III, and IV had molecular formulas of  $C_{33}H_{52}N_2O_6$  (572.3755),  $C_{35}H_{52}N_2O_6$  (596.3764),  $C_{35}H_{54}N_2O_6$  (598.3884), and  $C_{35}H_{56}N_2O_6$  (600.4149), respectively. Hydrolysis of a mixture of the four compounds with 1.2 N HCl in 80% methanol yielded six compounds. They were identified by mass spectrometry as 2,2-dimethyl-5-amino-6-(3'-amino-4'-hydroxybutyryl)-4-chromone (fusarochromanone, TDP-1), 2,2-dimethyl-5-amino-6-(3'-N-acetyl-4'-hydroxybutyryl)-4-chromone (3'-N-acetylfusarochromanone, TDP-2), and methyl esters of four fatty acids, including 14-methylpentadecanoic acid, 10,12-octadecadienoic acid, 9-octadecenoic acid, and 16-methylheptadecanoic acid. The fatty acid methyl esters were resolved by gas chromatography with a capillary column. The locations of double bonds in the fatty acyl moieties of compounds II and III were determined by the EI mass spectra of their TMSO derivatives. The structures of the four compounds were determined to be 3'-N-acetyl-4'-O-fatty acylated fusarochromanone. The mass spectral analysis was supported by  $^1H$  NMR and infrared spectral data.

## INTRODUCTION

Fusarochromanone, also called TDP-1, is a mycotoxin produced by isolates of *Fusarium equiseti* (previously identified as *F. graminearum*) (Lee et al., 1985; Xie et al., 1989; Krogh et al., 1989). This toxin induces tibial dyschondroplasia (TD) in broiler chick (Lee et al., 1985; Cook et al., 1987) and reduces hatchabilities of fertile chicken eggs under experimental conditions (Lee et al., 1985). TD is a common cartilage abnormality in broiler chickens, turkeys, and ducks and has been found all over the world (Hemsly, 1970; Laursen-Jones, 1970; Siller and Duff, 1970; Poulos, 1978; Shane, 1979; Burton et al., 1981). Recently this toxin was found in cereal feed associated with field cases of TD in chickens in Denmark (Krogh et al., 1989).

Fusarochromanone is a chromone derivative with an amino group at C5 and a 4-carbon side chain at C6. The substitution pattern in this compound is thought to be unique. It is the first reported naturally occurring chromone with an amino substituent at C5. The placement of the side chain at C6 is also unique (Pathre et al., 1986). The chemical name is 2,2-dimethyl-5-amino-6-(3'-amino-4'-hydroxybutyryl)-4-chromone. The compound exhibits blue fluorescence under UV light.

The 3'-N-acetyl derivative of fusarochromanone with a trivial name TDP-2 was also found in cultures of *F. equiseti* (Xie et al., 1989). Figure 1 shows structures of fusarochromanone and 3'-N-acetylfusarochromanone. In this paper we report the production and structure identification of four fatty acid esters of fusarochromanone from rice cultures of *F. equiseti*.

## MATERIALS AND METHODS

**Culture Preparation.** Rice media were prepared according to the method described by Xie et al. (1989). The media were inoculated with plugs of potato dextrose agar (PDA) cultures of *F. equiseti* (Alaska 2-2). The rice cultures were incubated at room temperature for 4 weeks with daily shaking for the first 5 days to provide uniform mycelium growth. At 4 weeks cultures were harvested, dried in a ventilation hood, and ground into a fine flourlike consistency for extraction.

**Extraction and Isolation.** Two hundred fifty grams of ground rice culture was doubly extracted with 1 L of ethyl acetate

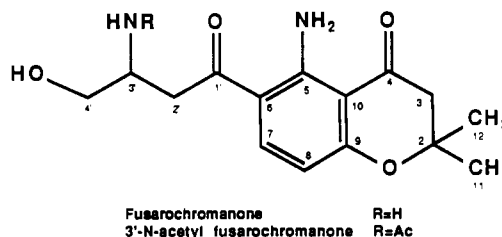


Figure 1. Structure of fusarochromanone (TDP-1) and 3'-N-acetylfusarochromanone (TDP-2).

in a 2-L flask. The extract was filtered through Whatman No. 4 filter paper and then evaporated to dryness in vacuo. The residue was dissolved in ca. 10 mL of ethyl acetate and applied to a Florisil (100 mesh, Fisher) column (4.5 × 50 cm). The column was eluted with 1 L of petroleum ether (boiling point 60–70 °C), 1 L of ethyl acetate–acetone (9:1), and 1 L of ethyl acetate–acetone (4:1). Each 100 mL of ethyl acetate–acetone fraction was collected, evaporated to dryness, and redissolved in 4 mL of chloroform. One microliter of the solution from each fraction was spotted onto silica gel plates (40 × 80 mm, 0.25-mm thickness, plastic sheet; Macherey-Nagel, Duren, Germany). The plates were developed in chloroform–methanol (49:1) and illuminated with long-wavelength UV light. Fractions showing a blue fluorescent spot with  $R_f$  value of 0.47 were combined and applied onto preparative thin-layer chromatography (TLC) plates made in our laboratory with TLC grade silica gel (Aldrich). The plates were developed in chloroform–methanol (97:3), and the fluorescent band was scraped under UV light. Silica gel containing the band was extracted with acetone and filtered through Whatman No. 42 filter paper. The preparative TLC was repeated until a preparation showing a single spot on the analytical TLC plate was obtained. This TLC preparation was called TDP-9 for convenience. A total of 3 kg of the rice culture was extracted.

**Hydrolysis of TDP-9.** About 1 mg of TDP-9 was hydrolyzed with 1 mL of 1.2 N HCl in 80% methanol at room temperature for 18 h. The reaction mixture was neutralized by adding concentrated  $NH_4OH$ . The reaction solution was then evaporated to dryness in  $N_2$ , and the residue was washed with 1 mL of chloroform. Five microliters of the chloroform solution was applied to a 40 × 80 mm TLC plate and developed in chloroform–methanol (99:1). The plate was checked under UV light and then soaked in 10%  $H_3PO_3MoO_4$  in EtOH followed by heating on a hot plate. Three components were found in the reaction mixture and called HY1, HY2, and HY3. HY1 and HY2 showed

blue fluorescence under UV light. HY3 was not fluorescent but showed dark blue color after  $H_3PO_3MoO_4$  treatment. They were isolated by scraping and eluting the corresponding bands from  $40 \times 80$  mm TLC plates chromatographed in the above developing system.

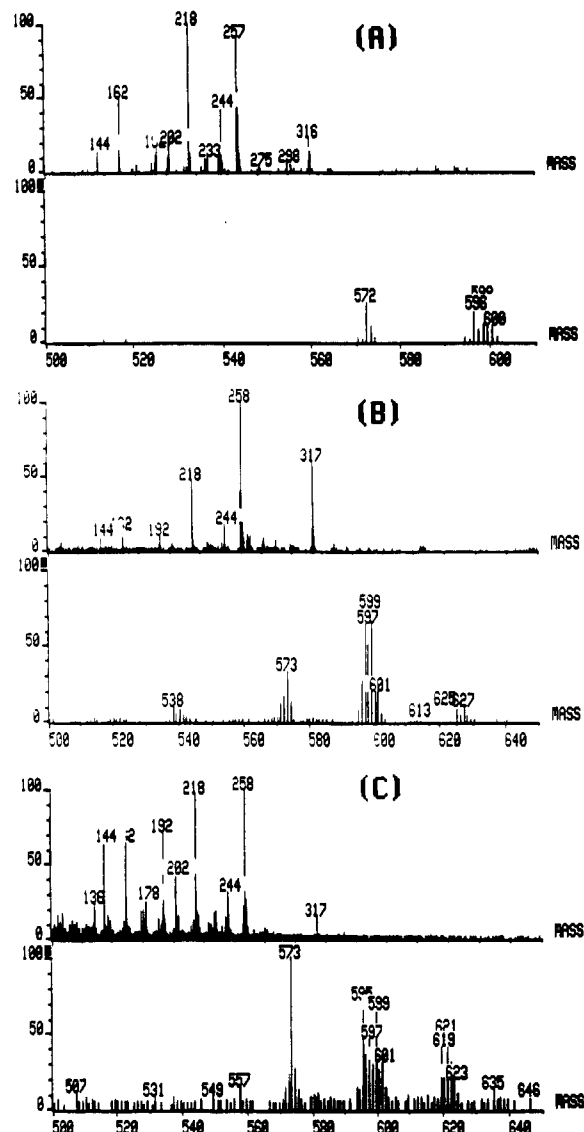
**Preparation of Trimethylsilyloxy Derivatives of Fatty Acid Methyl Esters.** Three milligrams of TDP-9 was hydrolyzed according to the method described above. Trimethylsilyloxy (TMSO) derivatives of methyl esters of the unsaturated fatty acids obtained from the hydrolysis of TDP-9 were prepared by hydroxylation of the olefinic bond by oxidation with  $OsO_4$  and subsequent reduction of the osmates with  $Na_2SO_3$ , generally following the procedure described by Cavalli et al. (1978). The hydrolysis product was dissolved in 2 mL of pyridine-dioxane (1:8) and treated with 20 mg of  $OsO_4$  (2% solution in dioxane). After the mixture was allowed to stand for 2 h, 15 mL of  $Na_2SO_3$  suspension (85 mL of 16%  $Na_2SO_3$  solution in 25 mL of methanol) was added. The mixture was allowed to stand for a further 2 h and filtered through glass wool. The filtrate was evaporated in  $N_2$  to dryness, and the residue was dissolved in 2 mL of dichloromethane. The dichloromethane solution was dried on anhydrous sodium sulfate, evaporated to dryness, and redissolved in 0.5 mL of dichloromethane. Fifty microliters of the dichloromethane solution was evaporated and reacted with 50  $\mu$ L of Tri Sil/TBT (Pierce) at room temperature for 20 min. The reaction mixture containing TMSO derivatives of the fatty acid methyl esters (FAME) was ready for GC-MS analysis.

**Synthesis of Fatty Acid Esters of 3'-N-Acetyl Derivative of Fusarochromanone.** Eight milligrams of 3'-N-acetylfusarochromanone was reacted with 20 mg of oleic anhydride (C18:1, *cis*-9-octadenoic anhydride) (Sigma) in 2 mL of pyridine at 65  $^{\circ}C$  for 3 h. The reaction solution was evaporated to dryness in  $N_2$ . The residue was dissolved in 1 mL of  $CHCl_3$ . The reaction product, which is fluorescent under UV light, was isolated by preparative TLC using chloroform-methanol (97:3) as the developing system. Two other fatty acid esters were made by reacting 8 mg of 3'-N-acetylfusarochromanone with 20 mg of elaidic anhydride (C18:1, *trans*-9) (*trans*-9-octadecenoic anhydride) (Sigma) and 7 mg of 3'-N-acetylfusarochromanone with 20  $\mu$ L of linoleic anhydride (C18:2, [*cis*,*cis*]-9,12) (Sigma) in 1.5 mL of pyridine at the same conditions and isolated with the same method.

**Spectral Analysis.** Low-resolution (1000) electron impact (EI), high-resolution (10 000) EI, low-resolution positive chemical ionization (PCI), fast atom bombardment (FAB) mass spectra of TDP-9 and low-resolution (1000) EI mass spectra of HY1, HY2, and the synthesized fatty acyl derivatives of 3'-N-acetylfusarochromanone were obtained by direct inlet on a VG 7070 EQ mass spectrometer. EI gas chromatography-mass spectrometry (GC-MS) data of HY3 were obtained on the VG mass spectrometer coupled with an HP 5890 gas chromatograph. A DB-5 capillary column (15-m length) was used. The temperature program was 80 to 280  $^{\circ}C$ , 1  $^{\circ}C$ /min. GC-MS data of TMSO derivatives of FAME yielded from the hydrolysis of TDP-9 were also obtained on the same instrument. The GC temperature program was 80  $^{\circ}C$  for 1 min and raised to 280  $^{\circ}C$  at a rate of 5  $^{\circ}C$ /min. All the EI mass spectra were obtained at 70 eV. Methane was used as the reactant gas in PCI mode. Thioglycerol was used as the matrix in FAB mode.  $^1H$  NMR spectra of TDP-9 and the synthetic linoleic acid and elaidic acid derivatives of 3'-N-acetylfusarochromanone were obtained in 100%  $CDCl_3$  on a Bruker AC-200 NMR spectrometer operating at 200 MHz. The  $^1H$  NMR spectrum of the synthetic oleic acid derivative of 3'-N-acetylfusarochromanone was obtained in 100%  $CDCl_3$  on a Nicolet NT300 WB FT-NMR spectrometer operating at 300 MHz. Infrared (IR) spectra of TDP-9 and the synthetic elaidic and linoleic acid derivatives of 3'-N-acetylfusarochromanone were obtained in chloroform solution on a Nicolet 510P FT-IR spectrometer (Nicolet Analytical Instrument, Madison, WI).

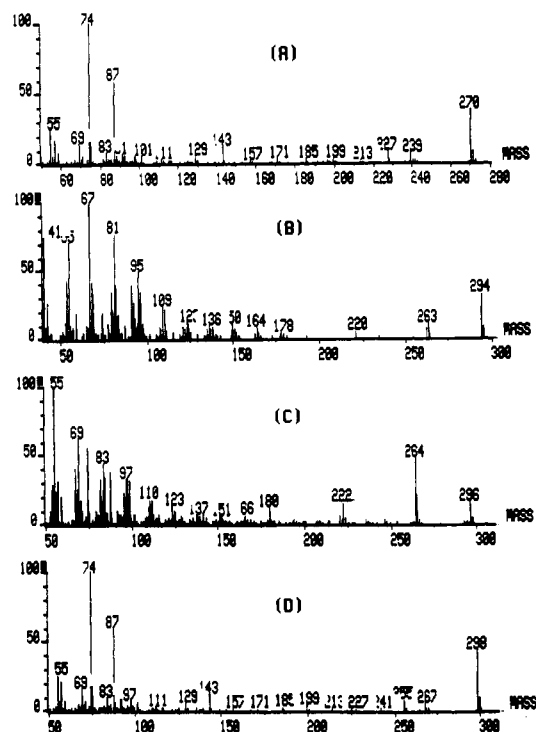
## RESULTS AND DISCUSSION

The low-resolution (EI) mass spectrum of TDP-9 is shown in Figure 2A. The low-mass region of the spectrum is very similar to those of fusarochromanone and 3'-N-acetylfusarochromanone (Xie et al., 1989). They all have



**Figure 2.** Mass spectra of TDP-9 containing compounds I-IV. (A) Low-resolution EI spectrum showing  $M^+$  at  $m/z$  572, 596, 598, and 600 for compounds I, II, III, and IV, respectively. (B) PCI spectrum with methane as reactant gas showing quasi-molecular ions at  $m/z$  573 ( $M + H$ ) $^+$  and 601 ( $M + C_2H_5$ ) $^+$  for compound I, 597 ( $M + H$ ) $^+$  and 625 ( $M + C_2H_5$ ) $^+$  for compound II, 599 ( $M + H$ ) $^+$  and 627 ( $M + C_2H_5$ ) $^+$  for compound III, and 601 ( $M + H$ ) $^+$  for compound IV. (C) FAB spectrum with thioglycerol as the matrix showing quasi-molecular ions at  $m/z$  573 ( $M + H$ ) $^+$  and 595 ( $M + Na$ ) $^+$  for compound I, 597 ( $M + H$ ) $^+$ , 619 ( $M + Na$ ) $^+$ , and 635 ( $M + K$ ) $^+$  for compound II, 599 ( $M + H$ ) $^+$  and 621 ( $M + Na$ ) $^+$  for compound III, and 601 ( $M + H$ ) $^+$  and 623 ( $M + Na$ ) $^+$  for compound IV.

a base peak at  $m/z$  218 which results from an  $\alpha$  cleavage between  $C1'$  and  $C2'$ . Other fragment ions at  $m/z$  257, 244, 233, 202, and 162 have been found in the EI mass spectra of the three compounds. Fragment ion at  $m/z$  316 which is ( $M - H_2O$ ) $^+$  in the mass spectrum of 3'-N-acetylfusarochromanone is also displayed in the spectrum of TDP-9. This strongly suggests that TDP-9 is a derivative of fusarochromanone and mostly likely a derivative of 3'-N-acetylfusarochromanone. In the high-mass region, the spectrum displays ions at  $m/z$  572, 596, 598, and 600, indicating that the TDP-9 TLC preparation might contain four compounds and the above ions are their molecular ions. They are called compounds I, II, III, and IV, respectively, in this paper. The existence of four compounds in the TLC preparation and their molecular weights are confirmed by PCI (Figure 2B) and FAB (Figure

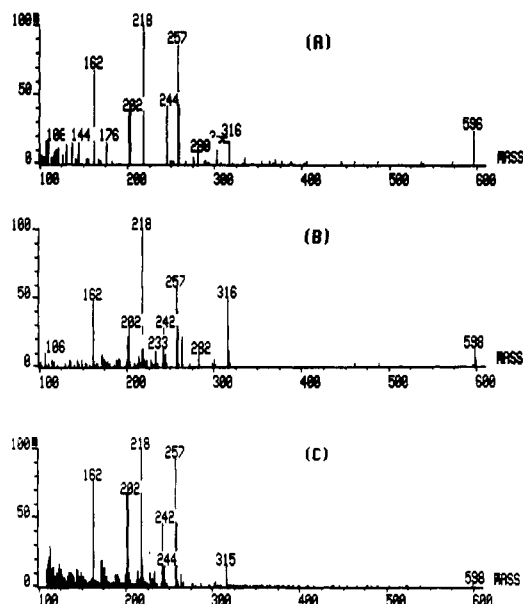


**Figure 3.** Low-resolution EI mass spectra of TDP-9 hydrolysis products HY3A (A) showing  $M^+$  at  $m/z$  270 and  $(M - OCH_3)^+$  at  $m/z$  239, HY3B (B) showing  $M^+$  at  $m/z$  294 and  $(M - OCH_3)^+$  at  $m/z$  263, HY3C (C) showing  $M^+$  at  $m/z$  296 and  $(M - CH_3OH)^+$  at  $m/z$  264, and HY3D (D) showing  $M^+$  at  $m/z$  298 and  $(M - OCH_3)^+$  at  $m/z$  267.

2C) mass spectra. The PCI spectra displayed quasi-molecular ions at  $m/z$  573 ( $M + H$ ) $^+$  for compound I, 597 ( $M + H$ ) $^+$  and 625 ( $M + C_2H_5$ ) $^+$  for compound II, and 599 ( $M + H$ ) $^+$  and 627 ( $M + C_2H_5$ ) $^+$  for compound III. The ion at  $m/z$  601 might be  $(M + H)^+$  for compound IV or  $(M + C_2H_5)^+$  for compound I. The FAB spectrum displays quasi-molecular ions at  $m/z$  573 ( $M + H$ ) $^+$  and 595 ( $M + Na$ ) $^+$  for compound I; 597 ( $M + H$ ) $^+$ , 619 ( $M + Na$ ) $^+$ , and 635 ( $M + K$ ) $^+$  for compound II; 599 ( $M + H$ ) $^+$  and 621 ( $M + Na$ ) $^+$  for compound III; and 601 ( $M + H$ ) $^+$  and 623 ( $M + Na$ ) $^+$  for compound IV. The high-resolution mass spectrum of TDP-9 yielded molecular ions at  $m/z$  527.3755 (calculated as 572.3825 for  $C_{33}H_{52}N_2O_6$ ) for compound I, 596.3764 (calculated as 596.3825 for  $C_{35}H_{52}N_2O_6$ ) for compound II, 598.3884 (calculated as 598.3981 for  $C_{35}H_{54}N_2O_6$ ) for compound III, and 600.4149 (calculated as 600.4138 for  $C_{35}H_{56}N_2O_6$ ) for compound IV.

The EI mass spectra of hydrolysis products HY1 and HY2 indicate that the two compounds are fusarochromanone and 3'-N-acetylfusarochromanone, respectively. They are identical with the spectra of those two compounds we obtained previously (Xie et al., 1989).

GC-MS analysis indicated that there are four compounds in the HY3 TLC band. They are separated on GC by a capillary column and a slow-rising temperature program and are named HY3A, HY3B, HY3C, and HY3D, respectively. The four compounds are identified by mass spectrum library searching. The EI mass spectrum of HY3A (Figure 3A) displays a molecular ion at  $m/z$  270 and ion at  $m/z$  239 ( $M - OCH_3$ ) $^+$ . The spectrum matches that of 14-methylpentadecanoic acid methyl ester in the NBS mass spectrum library with a fitness of 90.4% (93.6% reverse). It also matches the spectrum of hexadecanoic acid methyl ester with a lower fitness of 84.9% (88.8% reverse). The EI spectrum of HY3B (Figure 3B) had a molecular ion at  $m/z$  294 and ion at  $m/z$  263 ( $M - OCH_3$ ) $^+$ .



**Figure 4.** Low-resolution EI mass spectra of the synthetic linoleic acid derivative (A), elaidic acid derivative (B), and oleic acid derivative of 3'-N-acetylfusarochromanone (C).

The spectrum matched the spectra of 10,13-octadecadienoic acid methyl ester and 11,14-octadecadienoic acid methyl ester with almost identical fitness (82.6%, 96.9% reverse, and 82.6%, 95.9% reverse). The EI spectrum of HY3C (Figure 3C) displays a molecular ion at  $m/z$  296 and ion at  $m/z$  264 ( $M - CH_3OH$ ) $^+$ . It matches the spectrum of 11-octadecenoic acid methyl ester with a fitness of 81.2% (96.1% reverse), and the spectra of methyl esters of four other octadecenoic acids, including 13-octadecenoic acid, 12-octadecenoic acid, 14-octadecenoic acid, and 9-octadecenoic acid, with slightly lower fitness. The EI spectrum of HY3D (Figure 3D) shows a molecular ion at  $m/z$  298 and ion at  $m/z$  267 ( $M - OCH_3$ ) $^+$ . It matched the spectra of 16-methylheptadecanoic acid methyl ester (83.4%, 88.6% reverse) and 15-methylheptadecanoic acid methyl ester (83.0%, 90.6% reverse).

Figure 4 shows the EI mass spectra of the synthetic linoleic acid derivative of 3'-N-acetylfusarochromanone with a molecular ion at  $m/z$  596 (A), elaidic acid derivative of 3'-N-acetylfusarochromanone with a molecular ion at  $m/z$  598 (B), and oleic acid derivative of 3'-N-acetylfusarochromanone (C). They are almost identical with that of TDP-9 except that the latter shows the molecular ions of four compounds.

The above evidence indicates that compounds I-IV are fatty acid esters of 3'-N-acetylfusarochromanone. They were hydrolyzed to 3'-N-acetylfusarochromanone, fusarochromanone, and the corresponding fatty acids, which were subsequently methylated in the presence of methanol and HCl.

The mass spectral analysis is supported by the  $^1H$  NMR data of TDP-9 ( $\delta$ ,  $CDCl_3$ ): C2(CH $_3$ ) $_2$ , 1.45, s, 6 H; C3H $_2$ , 2.96, s, 2 H; C5NH $_a$ , 9.37-9.48, b s, 1 H; C5NH $_b$ , 9.48-9.60, b s, 1 H; C7H, 7.38, d,  $J = 9$  Hz, 1 H; C8H, 6.07, d,  $J = 9$  Hz, 1 H; C2'Ha, 3.00, dd,  $J = 5, 16$  Hz, 1 H; C2'Hb, 3.42, dd,  $J = 5, 16$  Hz; C3'H, 4.47-4.65, m, 1 H; C3'NH, 6.38, d,  $J = 8$  Hz, 1 H; NHAc, 1.97, s, 3 H; C4'Ha, 4.14, dd,  $J = 5, 11$  Hz, 1 H; C4'Hb, 4.34, dd,  $J = 5, 11$  Hz, 1 H; COCH $_2$ CH $_2$ , 2.28, t,  $J = 7, 2$  H; CH $_2$ CH=CHCH $_2$ , 5.24-5.44, m; CH $_2$ CH=CHCH $_2$ , 1.45-1.71, m; CH $_2$ CH $_3$ , 0.80-0.92, m, 3 H; (CH $_2$ ) $_n$ CH $_3$ , 1.16-1.45, m. Most of the signals have been found in the  $^1H$  spectrum of 3'-N-acetylfusarochromanone (Xie et al., 1989). The last five signals

above, which were not found in the spectrum of 3'-*N*-acetylfusarochromanone, arise from the fatty acyl moieties. The esterification of the hydroxy at C4' resulted in a downfield shift from 3.70 as a multiplet to 4.14–4.34 as double doublet from a comparison of the spectra of 3'-*N*-acetylfusarochromanone and TDP-9. The synthesized linoleic acid derivative of 3'-*N*-acetylfusarochromanone gives the following  $^1\text{H}$  NMR spectrum ( $\delta$ ,  $\text{CDCl}_3$ ): C2( $\text{CH}_3$ )<sub>2</sub>, 1.45, s, 6 H; C3H<sub>2</sub>, 2.70, s, 2 H; C5NH<sub>a</sub>, 9.34–9.48, b, 1 H; C5NH<sub>b</sub>, 9.48–9.62, b, 1 H; C7H, 7.83, d,  $J = 9$  Hz, 1 H; C8H, 6.07, d,  $J = 9$  Hz, 1 H; C2'Ha, 3.60, dd,  $J = 6, 16$  Hz, 1 H; C2'Hb, 3.25, dd,  $J = 5, 16$  Hz, 1 H; C3'H, 4.47–4.65, m, 1 H; C3'NH, 6.39, d,  $J = 8.3$  Hz, 1 H; NHAc, 1.97, s, 3 H; C4'Ha, 4.15 dd,  $J = 5, 11$  Hz, 1 H; C4'Hb, 4.34, dd,  $J = 6, 11$  Hz, 1 H; COCH<sub>2</sub>CH<sub>2</sub>, 2.29, t,  $J = 7$  Hz, 2 H; CH<sub>2</sub>CHCHCH<sub>2</sub>-CHCHCH<sub>2</sub>, 5.20–5.45, m, 4 H; CH<sub>3</sub>CH<sub>2</sub>, 0.88, t,  $J = 7$  Hz, 3 H; (CH<sub>2</sub>)<sub>n</sub>, 1.07–1.49, m, 16 H; CH<sub>2</sub>CHCHCH<sub>2</sub>CHCHCH<sub>2</sub>, 1.49–1.74, m, 4 H; CHCHCH<sub>2</sub>CHCH, 1.98–2.13, m, 2 H. The synthesized elaidic acid derivative of 3'-*N*-acetylfusarochromanone gives the following  $^1\text{H}$  NMR spectrum ( $\delta$ ,  $\text{CDCl}_3$ ): C2( $\text{CH}_3$ )<sub>2</sub>, 1.45, s, 6 H; C3H<sub>2</sub>, 2.70, s, 2 H; C5NH<sub>a</sub>, 9.32–9.48, b, 1 H; C5NH<sub>b</sub>, 9.48–9.56, b, 1 H; C7H, 7.83, d,  $J = 9$  Hz, 1 H; C8H, 6.07, d,  $J = 9$  Hz, 1 H; C2'Ha, 3.00, dd,  $J = 6, 16$  Hz; C2'Hb, 3.25, dd,  $J = 5, 16$  Hz, 1 H; C3'H, 4.47–4.63, m, 1 H; C3'NH, 6.38, d,  $J = 8$  Hz, 1 H; NHAc, 1.97, s, 3 H; C4'Ha, 4.14, dd,  $J = 5, 11$  Hz, 1 H; C4'Hb, 4.34, dd,  $J = 6, 11$  Hz, 1 H; COCH<sub>2</sub>CH<sub>2</sub>, 2.29, t,  $J = 7$  Hz, 2 H; CH<sub>2</sub>CHCHCH<sub>2</sub>, 5.27–5.42, m, 2 H; CH<sub>3</sub>CH<sub>2</sub>, 0.88, t,  $J = 6$  Hz, 3 H; (CH<sub>2</sub>)<sub>n</sub>, 1.07–1.54, m, 22 H; CH<sub>2</sub>CHCHCH<sub>2</sub>, 1.54–1.88, m, 4 H. The synthetic oleic acid derivative of 3'-*N*-acetylfusarochromanone gives the following  $^1\text{H}$  NMR spectrum ( $\delta$ ,  $\text{CDCl}_3$ ): C2( $\text{CH}_3$ )<sub>2</sub>, 1.43, s, 6 H; C3H<sub>2</sub>, 2.68, s, 2 H; C5NH<sub>a</sub>, 9.35–9.48, b, 1 H; C5NH<sub>b</sub>, 9.48–9.58, b, 1 H; C7H, 7.81, d,  $J = 9$  Hz, 1 H; C8H, 6.03, d,  $J = 9$  Hz, 1 H; C2'Ha, 3.22, dd,  $J = 5, 16$  Hz, 1 H; C2'Hb, 2.99, dd,  $J = 6, 16$  Hz, 1 H; C3'H, 4.46–5.01, m, 1 H; C3'NH, 6.43, d,  $J = 8$  Hz, 1 H; NHAc, 1.96, s, 3 H; C4'Ha, 4.13, dd,  $J = 5, 11$  Hz, 1 H; C4'Hb, 4.32, dd,  $J = 6, 11$  Hz, 1 H; COCH<sub>2</sub>-CH<sub>2</sub>, 2.27, t,  $J = 8$  Hz, 2 H; CH<sub>2</sub>CHCHCH<sub>2</sub>, 5.26–5.45, m, 2 H; CH<sub>3</sub>CH<sub>2</sub>, 0.84, t,  $J = 6$  Hz, 3 H; (CH<sub>2</sub>)<sub>n</sub>, 1.17–1.48, m, 22 H; CH<sub>2</sub>CHCHCH<sub>2</sub>, 1.50–1.67, m, 4 H. Those three spectra are very similar to that of TDP-9.

Additional supporting evidence for the mass spectral analysis was obtained from the IR spectral data of TDP-9 and the two synthesized fatty acid esters of 3'-*N*-acetylfusarochromanone. The TDP-9 IR spectrum shows absorption bands at 3395, 3285 (NH<sub>2</sub>, NH), 2930, 2856 (CH<sub>2</sub>), 1753 (C=O ester), 1657, 1597, and 1568 (C=O conjugated, amide II, Ar) cm<sup>-1</sup>. The IR spectra of the synthetic fatty acid esters of 3'-*N*-acetylfusarochromanone are almost identical with that of TDP-9. The structures of the four compounds in TDP-9 should be 3'-*N*-acetyl-4'-*O*-(fatty acyl)fusarochromanone.

In the mass spectrum of HY3 (Figure 3A) ion at  $m/z$  227 ( $M - \text{C}_3\text{H}_7$ )<sup>+</sup> resulting from the cleavage between C13 and C14 had a significantly higher relative intensity (7%) than ions at  $m/z$  213 ( $M - \text{C}_4\text{H}_9$ )<sup>+</sup> (<1%),  $m/z$  199 ( $M - \text{C}_5\text{H}_{11}$ )<sup>+</sup> (2%),  $m/z$  185 ( $M - \text{C}_6\text{H}_{13}$ )<sup>+</sup> (2%), and  $m/z$  171 ( $M - \text{C}_7\text{H}_{15}$ )<sup>+</sup> (2%). This fragmentation pattern indicates that there is a branch at C14. The branched-chain alkanes show enhanced bond breaking at the branching point. The increased fragmentation at these points has been ascribed to the increasing stability of primary, secondary, and tertiary carbonium ions. Although a branching at C12 could also result in a more intense ion at  $m/z$  217, it would also result in a more intense ion at  $m/z$  199. Such a phenomenon was not observed in the spectrum of HY3A. Thus, HY3A should be methyl

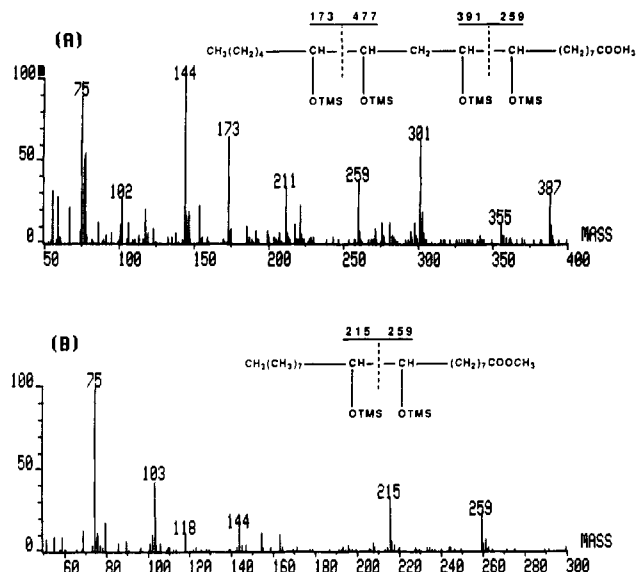


Figure 5. EI mass spectra of TMSO derivatives of fatty acid methyl esters HY3B (A) and HY3C (B) yielded from hydrolysis of TDP-9.

14-methylpentadecanoate, and the structure of compound I is established as 2,2-dimethyl-5-amino-6-[3'-*N*-acetyl-4'-*O*-(14-methylpentadecanoyl)butyryl]-4-chromone or 3'-*N*-acetyl-4'-*O*-(14-methylpentadecanoyl)fusarochromanone.

A similar fragmentation pattern was observed in the mass spectrum of HY3D. The relative intensity of ion at  $m/z$  255 ( $M - \text{C}_3\text{H}_7$ )<sup>+</sup> arising from the cleavage between C15 and C16 is 7%, which is significantly higher than that of ions at  $m/z$  241 ( $M - \text{C}_4\text{H}_9$ )<sup>+</sup> (<1%),  $m/z$  227 ( $M - \text{C}_5\text{H}_{11}$ )<sup>+</sup> (<1%),  $m/z$  213 ( $M - \text{C}_6\text{H}_{13}$ )<sup>+</sup> (1%),  $m/z$  199 ( $M - \text{C}_7\text{H}_{15}$ )<sup>+</sup> (3%), and  $m/z$  185 ( $M - \text{C}_8\text{H}_{17}$ )<sup>+</sup> (2%), indicating a branching point at C16. The relatively low intensity of ion at  $m/z$  241 excludes the possibility that the branching point is at C15. The spectrum of methyl 15-methylheptadecanoate ester in the mass spectrum library shows that both ions at  $m/z$  255 and 241 have higher intensities than other ions. HY3D is thus identified as methyl 16-methylheptadecanoate. The structure of compound IV is established as 2,2-dimethyl-5-amino-6-[3'-*N*-acetyl-4'-*O*-(16-methylheptadecanoyl)butyryl]-4-chromone or 3'-*N*-acetyl-4'-*O*-(16-methylheptadecanoyl)fusarochromanone.

Figure 5A shows the EI spectrum of TMSO derivatives of HY3B. The fragment ion at  $m/z$  173 results from the cleavage between C12 and C13, indicating that the original double-bond position is between C12 and C13. The fragment ion at  $m/z$  259 results from the cleavage between C9 and C10, indicating another original double-bond position is between C9 and C10. The fragment at  $m/z$  301 might come from loss of a TMSOH from ion at  $m/z$  391 (not observed). The fragment ion at  $m/z$  387 probably comes from loss of a TMSOH from ion at  $m/z$  477 (not observed). The fragment ion at  $m/z$  75 might be HOSi(CH<sub>3</sub>)<sub>2</sub>, and the fragment ion at  $m/z$  144 might be CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>COOCH<sub>3</sub>. HY3B is identified as 9,12-octadecadienoate. The structure of compound II is established as 2,2-dimethyl-5-amino-6-[3'-*N*-acetyl-4'-*O*-(9,12-octadecadienoyl)butyryl]-4-chromone or 3'-*N*-acetyl-4'-*O*-(9,12-octadecadienoyl)fusarochromanone.

Figure 5B shows the EI spectrum of TMSO derivative of HY3C. The fragments at  $m/z$  215 and 259 arise from the cleavage between C9 and C10 which bear the TMSO groups, indicating that the original double-bond position

in HY3C is between C9 and C10. Fragment at  $m/z$  103 is  $\text{CH}_2\text{OSi}(\text{CH}_3)_3$ . HY3C is thus identified as 9-octadecenoic acid methyl ester. The structure of compound III is established as 2,2-dimethyl-5-amino-6-[3'-*N*-acetyl-4'-*O*-(9-octadecenoyl)-4-chromone or 3'-*N*-acetyl-4'-*O*-(9-octadecenoyl)fusarochromanone.

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