

Falconensins A, B, C, and D, New Compounds Related to Azaphilone, from *Emericella falconensis*

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Four new compounds related to azaphilones, designated falconensins A (1), B (2), C (3), and D (4), were isolated from the mycelium of *Emericella falconensis*, a new ascomycetous fungus isolated from Venezuelan soil. The structures of falconensins A to D (1—4) were confirmed by spectroscopic investigation and chemical correlations. These falconensins are the hydrogenated azaphilones.

Keywords *Emericella falconensis*; azaphilone; falconensin A; falconensin B; falconensin C; falconensin D

A new ascomycetous fungus, *Emericella falconensis* HORIE, MIYAJI, NISHIMURA *et* UDAGAWA (anamorph; *Aspergillus falconensis* HORIE, MIYAJI, NISHIMURA *et* UDAGAWA) was isolated from Venezuelan soil in 1988.¹⁾ The mycelial dichloromethane extract of *E. falconensis*, strain NHL 2999 (=ATCC 76117), cultivated in Czapek medium supplemented with 0.2% yeast extract, had antibacterial activity against *Bacillus subtilis* COHN and antifungal activity against *Trichophyton mentagrophytes* (ROBIN) BLANCHARD. In the course of searching for antibacterial and/or antifungal metabolites, four new compounds designated falconensins A (1), B (2), C (3), and D (4) were isolated from the above extract. We now report the structural determination of 1, 2, 3, and 4.

Falconensins A (1), B (2), C (3), and D (4) showed positive coloration (green) in the Beilstein test, and gave molecular ion peaks at m/z 482, 484, and 486 (26:19:4), at m/z 484, 486, and 488 (15:10:2), at m/z 524, 526, and 528 (12:9:3), and at m/z 526, 528, and 530 (51:35:7), respectively, in the electron impact ionization (EI) mass spectra. The above results indicated that 1, 2, 3, and 4 possess two chlorine atoms in each molecule. Elemental analysis and/or high resolution mass spectrometry confirmed the molecular formulae of 1, 2, 3, and 4 as $C_{23}H_{24}Cl_2O_7$, $C_{23}H_{26}Cl_2O_7$, $C_{25}H_{26}Cl_2O_8$, and $C_{25}H_{28}Cl_2O_8$, respectively.

The infrared (IR) absorption of falconensin A (1) at 1735 cm^{-1} suggested the presence of an ester. On methanolysis with sodium methoxide using the same procedure as in the case of wortmin (5), an azaphilone-related compound, isolated from *Penicillium wortmannii* KLOCKER [= *Talaromyces wortmannii* (KLOCKER) C. R. BENJAMIN],²⁾ 1 gave only methyl dichlorodimethoxymethylbenzoate (6) as an isolable product. Compound 6 was

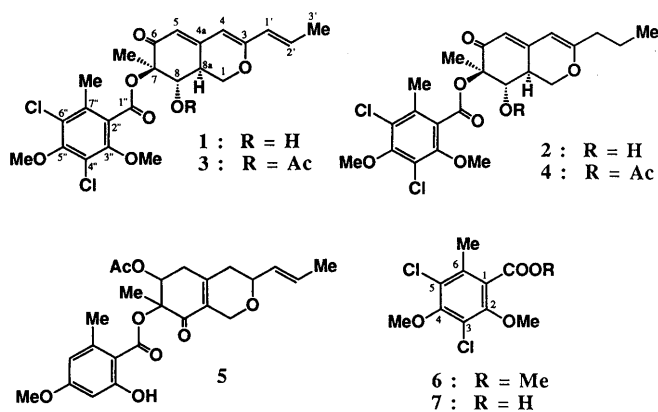
hydrolyzed by alkali to give the corresponding acid (7). The structures of 7 was confirmed as 3,5-dichloro-2,4-dimethoxy-6-methylbenzoic acid by comparison of the IR and proton nuclear magnetic resonance (¹H-NMR) spectra and by mixed fusion with an authentic sample, which was synthesized from ethyl dihydroorsellinate in four steps according to the literature.^{3,4)} These results confirmed the benzoate part structure of falconensin A (1).

The benzoate part ($C_{10}H_9Cl_2O_3$) of falconensin A (1) showed fragment ion peaks at m/z 247, 249, and 251 (98:64:11) in the EI mass spectrum, whereas the base peak was observed at m/z 235 ($C_{13}H_{15}O_4$) due to the remaining alcohol part. Decoupling experiments as well as the ¹H-NMR, the carbon-13 nuclear magnetic resonance (¹³C-NMR), and the ¹H-¹³C shift correlated (COSY) spectra of 1 (Table) were used to deduce the partial structure

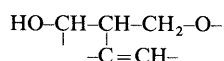
TABLE I. ¹H- and ¹³C-NMR Chemical Shift Correlations of Falconensin A (1) in CDCl₃

No.	δ_C (ppm)	δ_H (ppm)	J (Hz)	Correlated carbon signals with the proton on the same line in the COLOC spectrum
1	68.50 (Tdd) ^{a)}	3.84 (dd)	13.1, 10.7	
		4.80 (dd)	10.7, 5.2	
3	160.65 (m)			
4	102.68 (Dbrd)	5.57 (s)		C-3, C-5, C-1'
4a	150.41 (brs)			
5	116.50 (Ddd)	5.81 (d)	1.8	C-4, C-7
6	193.29 (d)			
7	86.53 (m)			
7-Me	16.86 (Qd)	1.49 (s)		C-6, C-7, C-8
8	70.06 (Dm)	4.72 (dd)	10.4, 3.1	
8-OH	—	2.79 (d)	3.1	
8a	38.05 (Ddd)	2.88 (dddd)	13.1, 10.4, 5.2, 1.8	
1'	125.41 (Ddd)	5.91 (dq)	15.6, 1.5	C-3
2'	134.03 (Dq)	6.47 (dq)	15.6, 7.0	
3'	18.38 (Qdd)	1.87 (dd)	7.0, 1.5	C-1', C-2'
1''	164.46 (s)			
2''	126.44 (q) ^{b)}			
3''	151.88 (q) ^{c)}			
3''-OMe	62.52 (Q) ^{d)}	3.90 (s) ^{b)}		C-3''
4''	120.66 (s)			
5''	154.32 (q) ^{c)}			
5''-OMe	60.64 (Q) ^{d)}	3.91 (s) ^{b)}		C-5''
6''	126.61 (q) ^{b)}			
7''	134.51 (q)			
7''-Me	17.21 (Q)	2.48 (s)		C-2'', C-6'', C-7''

a) The multiplicity is indicated. b, c, d) The assignments may be reversed.



of the alcohol part in **1**. When the vinylic proton at δ 6.47 (dq) was irradiated, the methyl protons at δ 1.87 (dd) and the vinylic proton at δ 5.91 (dq) were changed into a doublet and a broad singlet, respectively. Moreover, irradiation of the proton at δ 5.91 caused a change of the signals at δ 6.47 (q) and 1.87 (d). The above results suggested the presence of a 1-propenyl residue in the molecule of **1**. The double doublets at δ 4.80 and 3.84, which corresponded to the protons attached to carbon (δ 68.50) bearing an oxygen, and at δ 4.72, which corresponded to the proton attached to carbon (δ 70.06) bearing a hydroxy group, were all changed to doublets when the signal at δ 2.88 (dddd) was irradiated. This irradiation also caused a change of the vinylic proton at δ 5.81 from doublet to singlet. Therefore the presence of the partial structure



was confirmed in **1**. The remaining protons of the alcohol part of **1** were a vinylic proton at δ 5.57 and methyl protons at δ 1.49.

The correlation peaks in the ^1H - ^{13}C long-range shift correlated (COLOC) spectrum of **1** are listed in Table. The tertiary methyl protons at δ 1.49 were correlated with the conjugated carbonyl carbon at δ 193.29 (C-6), oxygen-bearing carbon at δ 86.53 (C-7), and the carbon bearing the hydroxy group at δ 70.06 (C-8). Correlation peaks of the vinylic proton at δ 5.81, which coupled allylically with the proton at δ 2.88 were observed with the carbons at δ 86.53 (C-7) and 102.68 (C-4). The other vinylic proton at δ 5.57 was correlated with the carbons at δ 116.50 (C-5), 160.65 (C-3), and 125.41 (C-1'). Furthermore, nuclear Overhauser enhancements (NOE) were observed on the vinylic protons at δ 5.91 and 5.81 when the vinylic proton at δ 5.57 was irradiated. From the above results, the structure of falconensin A (**1**) was concluded to be 7-(3,5-dichloro-2,4-dimethoxy-6-methylbenzoyl)-8-hydroxy-7-methyl-3-(1-propenyl)-1,7,8,8a-tetrahydro-6H-2-benzopyran-6-one.

Difference NOE experiments in conjunction with measurements of scalar coupling constants in the ^1H -NMR spectrum of **1** (Table) were conducted to determine the relative stereochemistry. When the proton signal of 8a-H (δ 2.88) was irradiated, NOE was observed at the tertiary methyl group at C-7 (δ 1.49) and one of the methylene protons at C-1 (δ 4.80). On the other hand, irradiation of the above methyl protons caused enhancement of the same proton at C-8a. These observations confirmed that the stereochemical relationship between the methyl at C-7 and the proton at C-8a is *cis* on the cyclohexenone ring. The large coupling constant (10.4 Hz) of 8-H (δ 4.72) and 8a-H indicated that these two protons have *trans*-diaxial configuration. Thus, the relative stereochemistry of falconensin A (**1**) was determined.

The ^1H -NMR spectra of falconensins **B** (**2**), **C** (**3**), and **D** (**4**) resemble those of falconensin A (**1**). The ultraviolet (UV) spectra of **1** and **3** showed maxima at 349 and 344 nm, respectively, whereas **2** and **4** both had a UV maximum at 323 nm. The above results suggested that **2** and **4** had one less conjugated double bond than **1** and **3**. Instead of the ^1H -NMR signals assigned to the 2-propenyl residue at C-3 [δ 1.87 (3H), 5.91 (1H), and 6.47 (1H) in **1**: δ 1.87 (3H),

5.90 (1H), and 6.45 (1H) in **3**], signals at δ 0.95 (3H), 1.60 (2H), 2.18 (1H), and 2.25 (1H) in **2** and those at δ 0.94 (3H), 1.60 (2H), 2.18 (1H), and 2.21 (1H) in **4** appeared. Hydrogenation of **1** and **3** on 5% Pd-C gave **2** and **4**, respectively, as the main products, which were identical with the natural products, including the circular dichroism (CD) curves. These results indicated that the side chain at C-3 in **2** and **4** is not a 2-propenyl residue, but a propyl residue. On acetylation, **1** and **2** gave quantitatively **3** and **4**, respectively, indicating the presence of one acetoxy group in **3** (δ 2.18) and **4** (δ 2.17). Since the proton attached at C-8 in **1** and **2** (δ 4.72 and 4.71, respectively) was shifted more than 1 ppm downfield in **3** and **4** (δ 6.09 and 6.07, respectively), it is clear that the acetylation occurred at the hydroxy group at C-8 in **1** and **2**. The relative structures of falconensins **B**, **C**, and **D** were thus confirmed as **2**, **3**, and **4**, respectively.

Falconensins **A** (**1**), **B** (**2**), **C** (**3**), and **D** (**4**) are hydrogenated azaphilones with a dichloroorseellinate moiety. These compounds (**1**—**4**) showed no antibacterial or antifungal activity against *B. subtilis* and *T. menta-grophytes*.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotation was measured with a JASCO DIP-181 spectrometer. EI-MS were taken with a JEOL JMD-D-300 spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^1H -NMR and ^{13}C -NMR spectra were recorded on a JEOL JNM-GX-400 spectrometer at 399.78 MHz and at 100.43 MHz, respectively, or ^1H -NMR spectra were taken with a JEOL JNM-GX-270 spectrometer at 270.17 MHz, using tetramethylsilane as an internal standard. The coupling patterns are indicated as follows: singlet = s, doublet = D or d, triplet = T or t, quartet = Q or q, multiplet = m, and broad = br. Capital letters refer to the pattern resulting from directly bonded coupling ($^1J_{\text{C,H}}$). CD curves were determined on a JASCO J-600 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low pressure liquid chromatography (LPLC) was performed with a Chemco Low-Prep 81-M-2 pump and a glass column (10 i.d. \times 150 or 200 mm) packed with Silica gel CQ-3 (30—50 μm , Wako). TLC was conducted on pre-coated Kieselgel 60 F₂₅₄ plates (Art. 5715, Merck). Spots on TLC plates were detected on the basis of their absorption of UV light.

Isolation of Falconensins from *E. falconensis* *E. falconensis*, strain NHL 2999, was cultivated in Czapek medium supplemented with 0.2% yeast extract (30 l) using 120 Roux flasks at 25 °C for 21 d. The dried mycelium (227 g) was extracted with CH_2Cl_2 and the organic layer was dried with Na_2SO_4 and then evaporated *in vacuo*. The extract obtained (17.9 g) was chromatographed on silica gel with benzene-AcOEt (20:1) to give the falconensins-containing fraction (3.0 g), and with benzene-AcOEt (2:1) to afford ergosterol. The former fraction was purified by repeated LPLC with hexane-acetone (40:1) to afford falconensin D (**4**) (12 mg) and falconensin C (**3**) (7 mg), with hexane-acetone (20:1) to afford falconensin B (**2**) (287 mg), and with hexane-acetone (10:1) to give falconensin A (**1**) (361 mg).

Falconensin A (**1**): Pale yellow needles with blue fluorescence, mp 84 °C (from hexane- CH_2Cl_2). Beilstein test: positive (green). $[\alpha]_{\text{D}}^{20} +238^\circ$ ($c=0.25$, MeOH). *Anal.* Calcd for $\text{C}_{23}\text{H}_{24}\text{Cl}_2\text{O}_7$: C, 57.15; H, 5.00. Found: C, 57.00; H, 5.03. EI-MS m/z (%): 482.0894 (M^+ , 482.0897 for $\text{C}_{23}\text{H}_{24}^{35}\text{Cl}_2\text{O}_7$, 26), 484.0873 ($\text{M}+2$, 484.0870 for $\text{C}_{23}\text{H}_{24}^{35}\text{Cl}^{37}\text{ClO}_7$, 19), 486.0845 ($\text{M}+4$, 486.0840 for $\text{C}_{23}\text{H}_{24}^{37}\text{Cl}_2\text{O}_7$, 4), 246.9930 (246.9929 for $\text{C}_{10}\text{H}_9\text{Cl}_2\text{O}_3$, 98), 249 (64), 251 (11), 235.0966 (235.0968 for $\text{C}_{13}\text{H}_{15}\text{O}_4$, 100). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 273 sh (3.76), 349 (4.57). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600—3200 (OH), 1735 (—COO—), 1665 (conjugated C=O). ^1H -NMR (CDCl_3) δ : 1.49 (3H, s, 7-Me), 1.87 [3H, dd, $J=7.0$, 1.5 Hz, 3'-H₃ (Me)], 2.48 (3H, s, 7''-Me), 2.79 (1H, d, $J=3.1$ Hz, 8-OH), 2.88 (1H, dddd, $J=13.1$, 10.4, 5.2, 1.8 Hz, 8a-H), 3.84 [1H, dd, $J=13.1$, 10.7 Hz, 1-H (ax.)], 3.90 (3H, s, 3'- or 5'-OMe), 3.91 (3H, s, 5''- or 3''-OMe), 4.72 (1H, dd, $J=10.4$, 3.1 Hz, 8-H), 4.80 [1H, dd, $J=10.7$, 5.2 Hz, 1-H (eq.)], 5.57 (1H, s, 4-H),

5.81 (1H, d, $J=1.8$ Hz, 5-H), 5.91 (1H, dq, $J=15.6$, 1.5 Hz, 1'-H), 6.47 (1H, dq, $J=15.6$, 7.0 Hz, 2'-H). CD ($c=2.1 \times 10^{-5}$, MeOH) $\Delta\epsilon^{20}$ (nm): -1.1 (206), +5.4 (225), -0.5 (244), +0.8 (276), -3.0 (320), +12.4 (366), +7.0 (385).

Falconensin B (2): Colorless prisms with blueish violet fluorescence, mp 130 °C (from MeOH). Beilstein test: positive (green), $[\alpha]_D^{20} +151^\circ$ ($c=0.25$, MeOH). EI-MS m/z (%): 484.1059 (M^+ , 484.1056 for $C_{23}H_{26}^{35}Cl_2O_7$, 15), 486.1017 ($M+2$, 484.1024 for $C_{23}H_{26}^{35}Cl^{37}ClO_7$, 10), 488.0995 ($M+4$, 488.0995 for $C_{23}H_{26}^{37}Cl_2O_7$, 2), 247 ($C_{10}H_9Cl_2O_3$, 100), 249 (64), 251 (12), 237 ($C_{13}H_{17}O_4$, 51). UV λ_{max}^{MeOH} nm (log ϵ): 323 (4.31). IR ν_{max}^{KBr} cm^{-1} : 3600—3400 (OH), 1735 (—COO—), 1670 (conjugated C=O). 1H -NMR ($CDCl_3$) δ : 0.95 [3H, t, $J=7.3$ Hz, 3'-H₃ (Me)], 1.49 (3H, s, 7-Me), 1.60 (2H, qt, $J=7.3$, 7.3 Hz, 2'-H), 2.18 (1H, dt, $J=13.7$, 7.3 Hz, 1'-H), 2.25 (1H, dt, $J=13.7$, 7.3 Hz, 1'-H), 2.49 (3H, s, 7''-Me), 2.82 (1H, d, $J=3.1$ Hz, 8-OH), 2.86 (1H, dddd, $J=13.1$, 10.4, 4.9, 1.8 Hz, 8a-H), 3.82 [1H, dd, $J=13.1$, 10.7 Hz, 1-H (ax.)], 3.90 (3H, s, 5''- or 3''-OMe), 3.91 (3H, s, 3''- or 5''-OMe), 4.71 (1H, dd, $J=10.4$, 3.1 Hz, 8-H), 4.77 [1H, dd, $J=10.7$, 4.9 Hz, 1-H (eq.)], 5.52 (1H, s, 4-H), 5.74 (1H, d, $J=1.8$ Hz, 5-H). ^{13}C -NMR ($CDCl_3$) δ : 13.57 (Qdt, C-3'), 16.85 (Qd, 7-Me), 17.16 (Q, 7''-Me), 20.02 (Tq, C-2'), 36.53 (Tm, C-1'), 37.73 (Dm, C-8a), 60.62 (Q, 3''- or 5''-Me), 62.47 (Q, 5''- or 3''-Me), 68.70 (Tdd, C-1), 70.12 (Dm, C-8), 86.47 (m, C-7), 100.82 (Ddt, C-4), 115.37 (Ddd, C-5), 120.66 (s, C-4''), 126.39 (q, C-2'' or -6''), 126.64 (q, C-6'' or -2''), 134.47 (q, C-7''), 150.38 (brs, C-4a), 151.91 (q, C-3'' or -C-5''), 154.29 (q, C-5'' or C-3''), 164.46 (s, C-1''), 168.45 (m, C-3), 193.56 (brs, C-6). CD ($c=2.7 \times 10^{-5}$, MeOH) $\Delta\epsilon^{20}$ (nm): -2.8 (212), +1.1 (227), -3.0 (307), +7.8 (342), +7.0 (352).

Falconensin C (3): Pale yellow crystalline powder with blue fluorescence, mp 152 °C (from hexane- CH_2Cl_2). Beilstein test: positive (green), $[\alpha]_D^{20} +301^\circ$ ($c=0.05$, MeOH). EI-MS m/z (%): 524.0997 (M^+ , 524.1003 for $C_{25}H_{26}^{35}Cl_2O_8$, 12), 526.0983 ($M+2$, 526.0976 for $C_{25}H_{26}^{35}Cl^{37}ClO_8$, 9), 528.0938 ($M+4$, 528.0944 for $C_{25}H_{26}^{37}Cl_2O_8$, 3), 247 ($C_{10}H_9Cl_2O_3$, 47), 249 (29), 251 (6), 235 ($C_{13}H_{15}O_4$, 100). UV λ_{max}^{MeOH} nm (log ϵ): 344 (4.39). IR ν_{max}^{KBr} cm^{-1} : 1740 (—COO—), 1660 (conjugated C=O). 1H -NMR ($CDCl_3$) δ : 1.57 (3H, s, 7-Me), 1.87 [3H, dd, $J=7.5$, 1.5 Hz, 3'-H₃ (Me)], 2.18 (3H, s, 8-OAc), 2.50 (3H, s, 7''-Me), 2.99 (1H, dddd, $J=13.4$, 11.0, 4.9, 2.4 Hz, 8a-H), 3.83 (3H, s, 3''- or 5''-OMe), 3.88 (3H, s, 5''- or 3''-OMe), 3.97 [1H, dd, $J=13.4$, 11.0 Hz, 1-H (ax.)], 4.34 [1H, dd, $J=11.0$, 4.9 Hz, 1-H (eq.)], 5.59 (1H, s, 4-H), 5.86 (1H, d, $J=2.4$ Hz, 5-H), 5.90 (1H, dq, $J=15.4$, 1.5 Hz, 1'-H), 6.09 (1H, d, $J=11.0$ Hz, 8-H), 6.45 (1H, dq, $J=15.4$, 7.5 Hz, 2'-H). CD ($c=2.3 \times 10^{-5}$, MeOH) $\Delta\epsilon^{20}$ (nm): +2.2 (217), -2.1 (241), +10.4 (359), +9.7 (371).

Falconensin D (4): Pale yellow crystalline powder with blueish violet fluorescence, mp 71 °C. Beilstein test: positive (green), $[\alpha]_D^{20} +196^\circ$ ($c=0.22$, MeOH). EI-MS m/z (%): 526.1153 (M^+ , 526.1159 for $C_{25}H_{28}^{35}Cl_2O_8$, 51), 528.1128 ($M+2$, 528.1130 for $C_{25}H_{28}^{35}Cl^{37}ClO_8$, 35), 530.1096 ($M+4$, 530.1101 for $C_{25}H_{28}^{37}Cl_2O_8$, 7), 247 ($C_{10}H_9Cl_2O_3$, 51), 249 (31), 251 (6), 235 ($C_{13}H_{17}O_4$, 100). UV λ_{max}^{MeOH} nm (log ϵ): 323 (4.23). IR ν_{max}^{NaCl} cm^{-1} : 1750 (—COO—), 1670 (conjugated C=O). 1H -NMR ($CDCl_3$) δ : 0.94 [3H, t, $J=7.3$ Hz, 3'-H₃ (Me)], 1.56 (3H, s, 7-Me), 1.60 (2H, tq, $J=7.5$, 7.3 Hz, 2'-H), 2.17 (3H, s, 8-OAc), 2.21 (1H, dt, $J=13.7$, 7.5 Hz, 1'-H), 2.25 (1H, td, $J=7.5$, 3.1 Hz, 1'-H), 2.50 (3H, s, 7''-Me), 2.93 (1H, dddd, $J=13.4$, 10.4, 5.5, 1.8 Hz, 8a-H), 3.81 (3H, s, 5''- or 3''-OMe), 3.88 (3H, s, 3''- or 5''-OMe), 3.91 [1H, dd, $J=13.4$, 11.0 Hz, 1-H (ax.)], 4.30 [1H, dd, $J=11.0$, 5.5 Hz, 1-H (eq.)], 5.54 (1H, s, 4-H), 5.78 (1H, d, $J=1.8$ Hz, 5-H), 6.07 (1H, d, $J=10.4$ Hz, 8-H). CD ($c=3.9 \times 10^{-5}$, MeOH) $\Delta\epsilon^{20}$ (nm): -2.5 (233), -2.0 (241), -1.0 (302), +3.9 (328), +8.1 (342), +7.0 (355).

Methanolysis of Falconensin A (1) Falconensin A (1) (45 mg) was dissolved in MeOH (2 ml), and NaOMe (11.8 mg) was added. The mixture

was refluxed for 20 min, then the solvent was evaporated *in vacuo*. The residue was acidified with dilute HCl and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and the solvent was removed by evaporation. The residue was purified by LPLC (benzene) to give methyl 3,5-dichloro-2,4-dimethoxy-6-methylbenzoate (6) (22 mg, 85%).

Compound 6: White amorphous powder. Beilstein test: positive (green). EI-MS m/z (%): 278 (M^+ , 45), 280 ($M+2$, 30), 282 ($M+4$, 6). UV λ_{max}^{MeOH} nm (log ϵ): 280 (3.28). IR ν_{max}^{KBr} cm^{-1} : 1715 (—COO—). 1H -NMR ($CDCl_3$) δ : 2.30 (3H, s, 6-Me), 3.88 (3H, s, OMe), 3.90 (3H, s, OMe), 3.94 (3H, s, OMe). ^{13}C -NMR ($CDCl_3$) δ : 17.40 (6-Me), 52.64 (COOMe), 60.61 (OMe), 62.16 (OMe), 121.20 (C-3), 127.00 (C-1 or -5), 128.32 (C-5 or -1), 133.37 (C-6), 152.39 (C-2 or -4), 154.14 (C-4 or -2), 166.90 (COOMe).

Hydrolysis of Methyl 3,5-Dichloro-2,4-dimethoxy-6-methylbenzoate (6) A 10% NaOH solution (2 ml) was added to a solution of 6 (30 mg) in MeOH (1 ml), and the mixture was left at 60 °C for 12 h. After cooling, the reaction mixture was acidified with 4 N HCl and extracted with $CHCl_3$, then the solvent was evaporated off *in vacuo*. The residue was crystallized from hexane- CH_2Cl_2 to give 3,5-dichloro-2,4-dimethoxy-6-methylbenzoic acid (7) (21 mg, 74%). This compound (7) was identical with an authentic specimen synthesized from ethyl dihydroorsellinate^{3,4)} on the basis of IR and 1H -NMR spectral comparison and mixed fusion.

3,5-Dichloro-2,4-dimethoxy-6-methylbenzoic Acid (7): Colorless needles, mp 135 °C (from hexane- CH_2Cl_2). Beilstein test: positive (green). EI-MS m/z (%): 264 (M^+ , 43), 266 ($M+2$, 27), 268 ($M+4$, 5). IR ν_{max}^{KBr} cm^{-1} : 3200—2400 (COOH), 1690 (COOH). 1H -NMR ($CDCl_3$) δ : 2.43 (3H, s, 6-Me), 3.92 (3H, s, OMe), 3.95 (3H, s, OMe).

Hydrogenation of Falconensins A (1) and C (3) with 5% Pd/C A suspension of 5% Pd/C (5 mg) in a solution of falconensin A (1) (60 mg) or C (3) (55 mg) in MeOH (5 ml) was stirred at room temperature in a hydrogen atmosphere for 7 min. After filtration to remove the catalyst, the solvent was evaporated off *in vacuo*. The residue was purified by LPLC using the solvent system of hexane-acetone (20:1) to afford dihydrofalconensin A (2) or D (4) (22 mg), which was identical with falconensins B and D, respectively, on the basis of 1H -NMR, UV, IR, and CD spectral comparison, TLC behavior, and mixed melting point determination.

Acetylation of Falconensins A (1) and B (2) Falconensin A (1) (47 mg) or B (2) (57 mg) was dissolved in a mixture of pyridine (0.5 ml) and Ac_2O (0.25 ml), and the solution was kept overnight at room temperature, then poured into ice-water and extracted with ether. The extract was dried over Na_2SO_4 . After evaporation of the solvent, the residue was purified by LPLC using the solvent system of hexane-acetone (40:1) to afford mono-*O*-acetylfalconensin A (3) (37 mg) or B (4) (38 mg), which was identical with the naturally occurring falconensin C or D, respectively, on the basis of 1H -NMR, UV, IR, and CD spectral comparison, TLC behavior, and mixed melting point determination.

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References

- 1) Y. Horie, M. Miyaji, K. Nishimura and S. Udagawa, *Trans. Mycol. Soc. Jpn.*, **30**, 257 (1989).
- 2) L. Merlini, R. Mondelli, G. Nasini and M. Hesse, *Helv. Chim. Acta*, **56**, 232 (1973).
- 3) L. Fitzpatrick, T. Sala and M. V. Sargent, *J. Chem. Soc., Perkin Trans. 1*, **1980**, 85.
- 4) G. B. Henderson and R. A. Hill, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 1111.