Isolation and Structures of New Azaphilone Derivatives, Falconensins E—G, from *Emericella falconensis* and Absolute Configurations of Falconensins A—G

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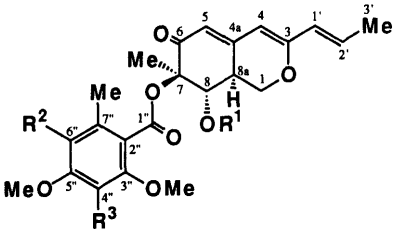
Three new azaphilone derivatives designated falconensins E (5), F (6), and G (7) were isolated from the mycelium of *Emericella falconensis*, along with falconensins A (1), B (2), C (3), D (4), and H (8), and three hopane-type triterpenes, zeorin (9), hopane-7β,22-diol (10), and hopane-6α,7β,22-triol (11). The structures of 5—7 were confirmed by spectroscopic investigation and chemical correlations. The absolute stereochemistry of falconensins A (1) to G (7) was also established.

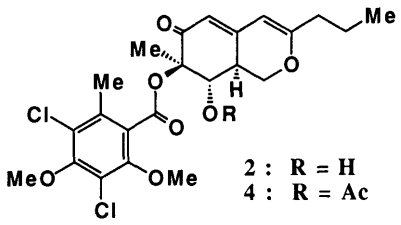
Key words *Emericella falconensis*; azaphilone; falconensin E; falconensin F; falconensin G; hopane-type triterpene

Recently we isolated four new hydrogenated azaphilones, falconensins A (**1**), B (**2**), C (**3**), and D (**4**),[1] and a new azaphilone, falconensin H (**8**),[2] from the dichloromethane extract of the mycelium of a new ascomycetous fungus, *Emericella falconensis* HORIE, MIYAJI, NISHIMURA and UDAGAWA, strain NHL 2999 (=ATCC 76117), found in Venezuelan soil in 1988.[3] All of the above compounds were also isolated from the mycelium of *E. fruticulosa* (RAPER and FENNELL) MALLOCH and CAIN, strain IFO 30841, the dichloromethane extract of which shows a thin layer chromatographic (TLC) pattern almost superimposable on that of *E. falconensis*. The inhibitory effect of **1**—**3** on 12-*O*-tetradecanoylphorbol 13-acetate inflammation in mice was recently reported (ID_{50} 1.1, 1.5, and 1.2 μmol/ear, respectively).[4] This result prompted us to investigate further the mycelial extract of *E. falconensis* and/or *E. fruticulosa*, leading to the isolation of three new azaphilone derivatives designated falconensins E (**5**), F (**6**), and G (**7**), and three hopane-type triterpenes, zeorin (hopane-6α,22-diol) (**9**), hopane-7β,22-diol (**10**), and hopane-6α,7β,22-triol (**11**). We now report the structural determination of **5**, **6**, and **7** and the determination of the absolute configurations of **1**—**7**.

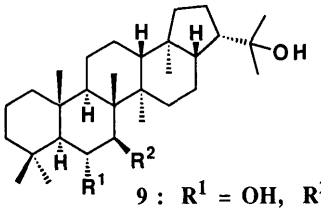
The relative structures of falconensin A—D (**1**—**4**) were confirmed in the previous paper.[1] The absolute configuration of **1**—**4** remained to be determined. On hydrogenation with 10% Pd–C for 2 h, **1** afforded a hexahydro derivative (**12**). Methanolysis of this compound



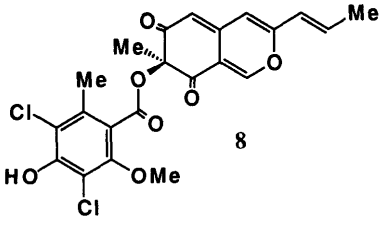
1 : R^1 = H, R^2 = R^3 =Cl
3 : R^1 = Ac, R^2 = R^3 = Cl
5 : R^1 = R^3 = H, R^2 = Cl
6 : R^1 = R^2 = R^3 = H
7 : R^1 = Ac, R^2 = R^3 = H



2 : R = H
4 : R = Ac



9 : R^1 = OH, R^2 = H
10 : R^1 = H, R^2 = OH
11 : R^1 = R^2 = OH



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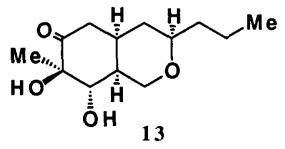

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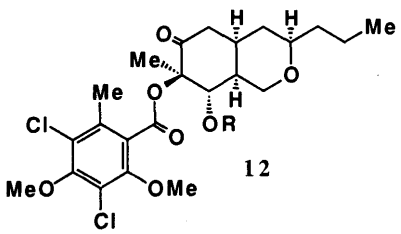

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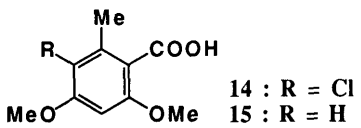

14 : R = Cl
15 : R = H

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gave a ketodiol (**13**). Decoupling experiments confirmed the assignments of the ^{1}H-NMR signals of **13**. The relationship between 8a-H (δ 1.85) and 8-H (δ 3.94) was determined as *trans*-diaxial from the coupling constant (J=11.6 Hz). Nuclear Overhauser enhancements (NOE) of 5.5 and 2.3%, respectively, were observed at 7-H_3 (δ 1.57) and one of the methylene protons at position 5 (δ 2.96), when 8a-H was irradiated. The above results confirmed that the conformation of cyclohexane ring was chair form. The above irradiation also revealed NOE at 4a-H (δ 2.35, 2.3%) and 1-H_2 (δ 3.50 and 4.36, 3.6 and 5.0%, respectively). From this fact and the coupling constant between the protons at 4a-H and 8a-H (5.0 Hz), it is clear that the juncture of the two six-membered rings is *cis*. The conformation of the tetrahydropyran ring was determined as chair form, because of the NOE (5.6%) between 3-H (δ 3.30) and 4a-H, and that (3.0%) between 3-H and one of the methylene proton at position 1 (δ 3.50), which was assigned to be axial. Therefore the relative structure and conformation of the ketodiol (**13**) was confirmed to be as shown in Fig. 1. A positive Cotton effect in the circular dichroism (CD) spectrum of **13** was observed at 286 nm. Based on the octant rule, the absolute configuration of **13** may be as shown in Fig. 1.

In order to confirm the absolute configuration of the ketodiol (**13**), the advanced Mosher's method[5] was applied to **13**. The (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters of **13** were synthesized and the values of the chemical shift differences between these (*R*)- and (*S*)-MTPA esters [$\Delta\delta=\delta_S-\delta_R$ in hertz (500 MHz)] were calculated. From the results (summarized in Fig. 2), the absolute stereochemistry at C-8 was determined as *S*. This result is consistent with that from the application of the octant rule to **13**, and the absolute structure of falconensin A (**1**) was consequently determined. It has already been confirmed that falconensins A (**1**)—D (**4**) have the same configuration by the comparison of derivatives, including CD curves.[1] The absolute structures of falconensins A—D are therefore as shown in **1**—**4**.

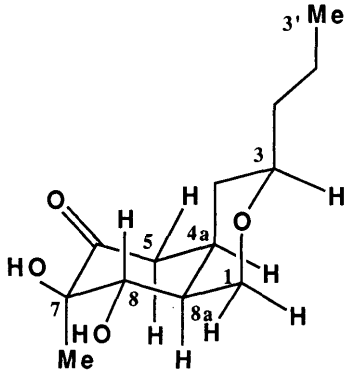

Fig. 1. Conformation of the Ketodiol (**13**)

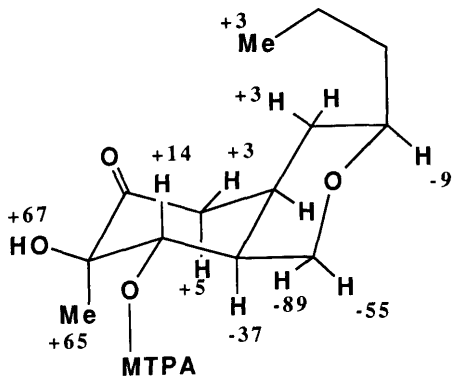

Fig. 2. Chemical Shift Differences ($\Delta\delta$ in Hertz) between (*R*)- and (*S*)-MTPA Esters of the Ketodiol (**13**)

The above conclusion is consistent with the exciton chirality rule[6] between the benzoate and the conjugated trienone or dienone: the first positive Cotton effect should be observed [**1**: 366 nm ($\Delta\varepsilon$+12.4), **2**: 342 (+7.8), **3**: 359 (+10.4), **4**: 342 (+8.1)] as shown in Table 1. But the second negative Cotton was not observed clearly owing to other complicated Cotton effects. Therefore, it appears that the absolute configurations of such hydrogenated azaphilones can be easily determined from the Cotton effect of the strongest and most bathochromic peak in the CD spectrum: a positive Cotton effect indicates *R*-configuration at C-7, whereas a negative one indicates *S*.

The molecular formulae of falconensins E (**5**) and F (**6**) were determined as $C_{23}H_{25}ClO_7$ and $C_{23}H_{26}O_7$, respectively, by high-resolution mass spectrometry. The fragment ion peak at *m/z* 235 ($C_{13}H_{15}O_4$) due to the azaphilone part was observed in the electron impact ionization (EI) mass spectra of **5** and **6**, as well as that of **1**.[1] The ^{1}H-NMR spectra of **5** and **6** were closely similar to that of **1**, except for the appearance of an aromatic proton at δ 6.40 in **5** and those at δ 6.32 and 6.36 in **6**. It was concluded that the azaphilone part of **5** and **6** was the same as that of **1**, including the relative stereochemistry, based on the analysis of the ^{13}C-NMR (Table 2) and ^{13}C–^{1}H long-range shift correlation (COLOC) spectra (Fig. 3), and decoupling experiments in the same manner as described in the previous paper.[1]

The fragment ion peaks of the benzoate part of **5** and **6** appeared at *m/z* 213 and 215 ($C_{10}H_{10}ClO_3$), and *m/z* 179 ($C_{10}H_{11}O_3$), respectively, in the EI mass spectra. These results indicated the lack of one or two chlorine atoms in the benzoate moiety of **5** and **6**, respectively, compared with **1**. In order to confirm the structures of the benzoate part, methanolysis with sodium methoxide followed by hydrolysis was performed using the same procedure as described in the previous papers.[1,2] 5-Chloro-2,4-dimethoxy-6-methylbenzoic acid (**14**)[7] and 2,4-dimethoxy-

Table 1. CD Values of Falconensins in MeOH

Compound	nm ($\Delta\varepsilon$)
1	385 (+7.0), 366 (+12.4), 320 (−3.0), 276 (+0.8), 244 (−0.5), 225 (+5.4), 206 (−1.1)
2	352 (+7.0), 342 (+7.8), 307 (−3.0), 227 (+1.1), 212 (−2.8)
3	371 (+9.4), 359 (+10.4), 241 (−2.1), 217 (+2.2)
4	342 (+8.1), 328 (+3.9), 302 (−1.0), 241 (−2.0), 233 (−2.5)
5	365 (+5.9), 312 (−1.3), 261 (+0.5), 234 (−2.0), 219 (+2.3)
6	366 (+6.0), 352 (+4.9), 296 (−0.9), 270 (+0.3), 248 (−0.8), 234 (−0.6), 216 (+1.5)
7	362 (+11.5), 306 (−1.1), 248 (−2.2), 215 (+4.2)

Table 2. ^{13}C-NMR Chemical Shifts of Falconensins in $CDCl_3$

No.	1	5	6	7
1	68.50	68.58	68.64	67.99
3	160.65	160.30	160.25	160.04
4	102.68	102.77	102.82	102.75
4a	150.41	149.88	149.82	148.73
5	116.50	116.77	116.86	117.19
6	193.29	193.40	193.81	193.18
7	86.53	85.58	85.14	82.38
7-Me	16.86	16.83	16.85	18.13
8	70.06	69.93	69.95	70.72
8-OCOCH$_3$				169.66
8-OCOCH$_3$				20.77
8a	38.05	37.87	37.84	38.16
1′	125.41	125.53	125.55	125.25
2′	134.03	133.61	133.55	133.77
3′	18.38	18.35	18.58	18.42
1″	164.46	165.23	165.97	166.23
2″	126.44[a]	117.44[a]	116.15	115.75
3″	151.88[b]	155.17[b]	157.63	158.67
3″-OMe	62.52[c]	56.34[c]	56.25	56.00
4″	120.66	94.41	96.54	96.38
5″	154.32[b]	156.69[b]	161.52	161.39
5″-OMe	60.64[c]	56.34[c]	55.40	55.28
6″	126.61[a]	116.10[a]	107.30	106.83
7″	134.51	136.71	139.47	139.62
7″-Me	17.21	17.26	19.66	19.87

a—*c*) The assignments may be reversed.

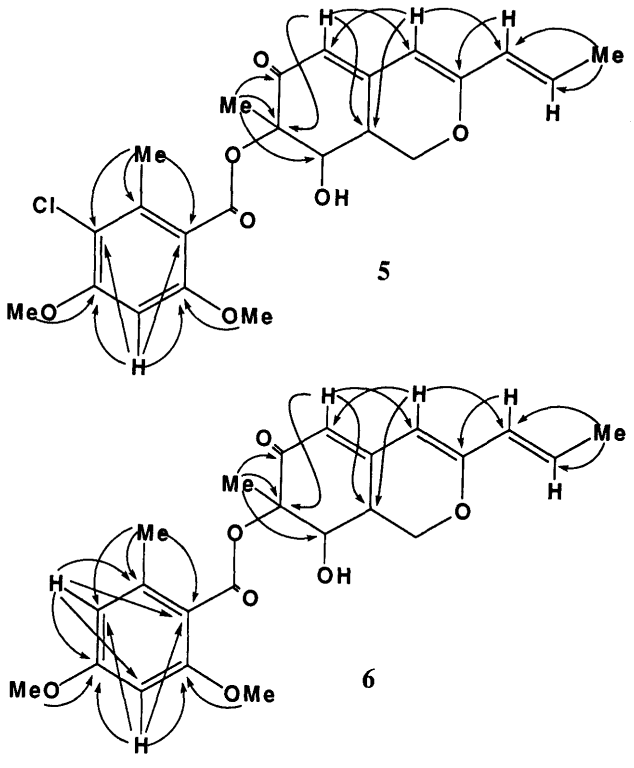

Fig. 3. COLOC Correlations of Falconensins E (**5**) and F (**6**)

An arrow indicates a COLOC correlation from the proton A to the carbon B ($H_A \rightarrow C_B$).

6-methylbenzoic acid (**15**)[8] were obtained from **5** and **6**, respectively. From the above results and the analyses of the COLOC spectra of **5** and **6**, the relative structures of falconensins E (**5**) and F (**6**) were confirmed.

Hydrogenation of **5** and **6** followed by methanolysis using the same procedure as in the case of **1** afforded a ketodiol (**13**), which was identical with the ketodiol derived from **1**, including the CD spectrum.[1] The Cotton effect of the strongest and most bathochromic peak in the CD spectrum was positive [**5**: 365 nm ($\Delta\varepsilon$+5.9), **6**: 366 (+6.0)], as shown in Table 1. Thus, the absolute stereochemistry at C-7 in **5** and **6** was determined as *R*, as in **1**—**4**. Therefore, the absolute structures of falconensins E and F were determined to be as shown in **5** and **6**.

The ^{1}H-NMR spectrum of falconensin G (**7**), $C_{25}H_{28}O_8$, closely resembled that of falconensin F (**6**), except for the appearance of the signals of an acetyl group (δ 2.15) and the downfield shift of 8-H (δ 6.11). On acetylation, **6** quantitatively gave **7**, which was identical with naturally occurring **7**, including the CD curves. Thus, the absolute structure of falconensin G is **7**. The Cotton effect of the strongest and most bathochromic peak in the CD spectrum of **7** was positive [**7**: 362 nm ($\Delta\varepsilon$+11.5)], as shown in Table 1.

Falconensins A—G (**1**—**7**) are hydrogenated azaphilones of a new type, having the same absolute stereochemistry. The absolute structures of these 1,8,8a-*O*-tetrahydroazaphilones are easily determined from the strongest and most bathochromic Cotton peak in the CD curves (a positive Cotton effect indicates *R*-configuration at C-7, whereas a negative one indicates *S*).

Zeorin (**9**) is a typical lichen metabolite,[9] and hopane-6α,7β,22-triol (**11**) has also been isolated from lichen.[10] Hopane-7β,22-diol (**10**) has not been found previously in lichen, but its acetate has.[11] The first example of a hopane-type triterpene without an oxygen function at C-3 to have been isolated from a fungus was dustanin (hopane-15α,22-diol) from *Aspergillus* sp. found on dust in air[12] and from a entomogenous fungus, *Aschersonia aleyrodis*.[13] It is interesting that these hopane-type triterpenes have been isolated as major components of free-living fungi in view of the relationship between *Emericella* sp. and lichen.

Experimental

General Procedure 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. ^{1}H-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, using tetramethylsilane as an internal standard. ^{1}H-NMR spectra for MTPA esters were recorded on a JEOL Lambda-500 spectrometer at 500.00 MHz. The coupling patterns are indicated as follows: singlet=s, doublet=d, triplet=t, quartet=q, multiplet=m, and broad=br. 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 on a Chemco Low-Prep 81-M-2 pump and glass column (10 i.d. × 200 mm) packed with Silica gel CQ-3 (30—50 μm; Wako). TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC plates were detected on the basis of their absorption of UV light.

Isolation of Metabolites from *Emericella falconensis* *E. falconensis*, strain NHL 2999, was cultivated for 21 d in Czapek medium supplemented with 0.2% yeast extract (30 l) using 120 Roux flasks at 25 °C. 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 obtained extract (17.9 g) was dissolved in $CHCl_3$ and the solution was filtered. The filtrate was concentrated by evaporation and chromatographed on

silica gel (400 g) with benzene–AcOEt (20:1) to obtain falconensins A—D (**1**—**4**),[2] with benzene–AcOEt (2:1), followed by purification by repeated LPLC with hexane–acetone (5:1), to afford falconensins E (**5**) (52 mg) and H (**8**) (6 mg), and with hexane–acetone (3:1) to give falconensins F (**6**) (62 mg) and G (**7**) (6 mg). The precipitates obtained from the mycelial CH_2Cl_2 extract was chromatographed on silica gel with benzene–AcOEt (2:1), followed by purification by repeated LPLC to give zeorin (**9**) (125 mg), hopane-7β,22-diol (**10**) (6 mg), and hopane-6α,7β,22-triol (**11**) (35 mg).

Falconensin E (**5**): Pale yellow needles, mp 94—95 °C (from hexane–CH_2Cl_2). Beilstein test: positive (green). $[\alpha]_D^{20}$ +299° (c=0.18, MeOH). EI-MS m/z (%): 448.1257 (M^+, 448.1287 for $C_{23}H_{25}{}^{35}ClO_7$, 49), 450.1253 (M+2, 450.1258 for $C_{23}H_{25}{}^{37}ClO_7$, 19), 235 ($C_{13}H_{15}O_4$, 16), 213 ($C_{10}H_{10}ClO_3$, 100), 215 (33). UV λ_{max}^{MeOH} nm (log ε): 349 (4.29). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1720 (–COO–), 1660 (conjugated C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.58 (3H, s, 7-Me), 1.87 [3H, dd, J=7.3, 1.5 Hz, 3′-H_3 (Me)], 2.46 (3H, s, 7″-Me), 2.76 (1H, d, J=2.8 Hz, 8-OH), 2.88 (1H, dddd, J=13.8, 10.4, 5.2, 2.4 Hz, 8a-H), 3.84 [1H, dd, J=13.8, 10.4 Hz, 1-H (ax.)], 3.88 (3H, s, 3″- or 5″-OMe), 3.92 (3H, s, 5″- or 3″-OMe), 4.75 (1H, dd, J=10.4, 2.8 Hz, 8-H), 4.77 [1H, dd, J=10.4, 5.2 Hz, 1-H (eq.)], 5.57 (1H, s, 4-H), 5.80 (1H, d, J=2.4 Hz, 5-H), 5.90 (1H, dq, J=15.3, 1.5 Hz, 1′-H), 6.40 (1H, s, 4″-H), 6.46 (1H, dq, J=15.3, 7.3 Hz, 2′-H). CD (c=4.0×10^{-5}, MeOH) $\Delta\varepsilon^{20}$ (nm): +2.3 (219), −1.0 (234), +0.5 (261), −1.3 (312), +5.9 (365). The assignments of ^{13}C-NMR signals are summarized in Table 2.

Falconensin F (**6**): Pale yellow needles, mp 73.5—74.5 °C (from hexane–CH_2Cl_2). $[\alpha]_D^{20}$ +288° (c=0.10, MeOH). EI-MS m/z (%): 414.1684 (M^+, 414.1679 for $C_{23}H_{26}O_7$, 29), 249 (64), 251 (12), 235 ($C_{13}H_{15}O_4$, 14), 179 ($C_{10}H_{11}O_3$, 100). UV λ_{max}^{MeOH} nm (log ε): 347 (4.24). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1720 (–COO–), 1650 (conjugated C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (3H, s, 7-Me), 1.87 [3H, dd, J=7.2, 1.8 Hz, 3′-H_3 (Me)], 2.42 (3H, s, 7″-Me), 2.87 (1H, dddd, J=13.1, 10.4, 5.5, 1.8 Hz, 8a-H), 2.90 (1H, d, J=2.4 Hz, 8-OH), 3.79 (3H, s, 5″-OMe), 3.80 (3H, s, 3″-OMe), 3.81 [1H, dd, J=13.1, 11.0 Hz, 1-H (ax.)], 4.73 (1H, dd, J=10.4, 2.4 Hz, 8-H), 4.77 [1H, dd, J=11.0, 5.5 Hz, 1-H (eq.)], 5.56 (1H, s, 4-H), 5.80 (1H, d, J=1.8 Hz, 5-H), 5.90 (1H, dq, J=14.7, 1.8 Hz, 1′-H), 6.32 (1H, d, J=1.8 Hz, 6″-H), 6.36 (1H, d, J=1.8 Hz, 4″-H), 6.46 (1H, dq, J=14.7, 7.2 Hz, 2′-H). CD (c=3.9×10^{-5}, MeOH) $\Delta\varepsilon^{20}$ (nm): +1.5 (216), −0.6 (234), −0.8 (248), +0.3 (270), −0.9 (296), +4.9 (352), +6.0 (366). The assignments of ^{13}C-NMR signals are summarized in Table 2.

Falconensin G (**7**): Pale yellow needles, mp 71 °C (from hexane–CH_2Cl_2). $[\alpha]_D^{20}$ +320° (c=0.10, MeOH). EI-MS m/z (%): 456.1781 (M^+, 456.1783 for $C_{25}H_{28}O_8$, 31), 235 ($C_{13}H_{15}O_4$, 41), 179 ($C_{10}H_{11}O_3$, 100). UV λ_{max}^{MeOH} nm (log ε): 349 (4.47). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1740 (–COO–), 1660 (conjugated C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.55 (3H, s, 7-Me), 1.87 [3H, dd, J=7.3, 1.8 Hz, 3′-H_3 (Me)], 2.15 (3H, s, 8-OAc), 2.44 (3H, s, 7″-Me), 2.96 (1H, dddd, J=13.1, 10.4, 4.9, 1.8 Hz, 8a-H), 3.76 (3H, s, 5″-OMe), 3.78 (3H, s, 3″-OMe), 3.93 [1H, dd, J=13.1, 11.0 Hz, 1-H (ax.)], 4.32 [1H, dd, J=11.0, 4.9 Hz, 1-H (eq.)], 5.57 (1H, s, 4-H), 5.86 (1H, d, J=1.8 Hz, 5-H), 5.89 (1H, dq, J=15.4, 1.8 Hz, 1′-H), 6.11 (1H, d, J=10.4 Hz, 8-H), 6.27 (1H, d, J=1.8 Hz, 6″-H), 6.30 (1H, d, J=1.8 Hz, 4″-H), 6.41 (1H, dq, J=15.4, 7.3 Hz, 2′-H). CD (c=6.8×10^{-5}, MeOH) $\Delta\varepsilon^{20}$ (nm): +4.2 (215), −2.2 (248), −1.1 (306), +11.5 (362). The assignments of ^{13}C-NMR signals are summarized in Table 2.

Hydrogenation of Falconensin A (1) with 10% Pd–C for 2 h Catalytic 10% Pd–C (5 mg) was suspended in a solution of falconensin A (**1**) (50 mg) in MeOH (3 ml) and the mixture was stirred at room temperature in a hydrogen atmosphere for 2 h. The catalyst was filtered off and the solvent was evaporated *in vacuo*. The residue was purified by LPLC with hexane–acetone (5:1) to afford hexahydrofalconensin A (**12**) (30 mg) as the main product.

Hexahydrofalconensin A (**12**): White amorphous powder. EI-MS m/z (%): 488 (M^+, $C_{23}H_{30}{}^{35}Cl_2O_7$, 34), 490 (M+2, 22), 492 (M+4, 8). UV λ_{max}^{MeOH} nm (log ε): 207 (4.47), 233 sh (3.87), 285 (2.74), 329 sh (2.01). IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (OH), 1740 (–COO–), 1720 (–CO–). ^{1}H-NMR ($CDCl_3$) δ: 0.89 [3H, t, J=7.0 Hz, 3′-H_3 (Me)], 1.11—1.44 (6H, m), 1.58 (3H, s, 7-Me), 1.91 (1H, br d, J=11.5 Hz, 8a-H), 2.31 (1H, m, 4a-H), 2.37 (1H, dd, J=16.2, 1.8 Hz, 5-H), 2.50 (1H, d, J=4.0 Hz, 8-OH), 2.51 (3H, s, 7″-Me), 2.85 (1H, dd, J=16.2, 6.7 Hz, 5-H), 3.36 (1H, m, 3-H), 3.56 (1H, dd, J=11.9, 2.2 Hz, 1-H), 3.90 (3H, s, 3″- or 5″-OMe), 3.92 (3H, s, 5″- or 3″-OMe), 4.38 (1H, br d, J=11.9 Hz, 1-H), 5.01 (1H, dd, J=11.5, 4.0 Hz, 8-H). CD (c=4.5×10^{-5}, MeOH) $\Delta\varepsilon^{20}$ (nm): +1.6 (288).

Methanolysis of Hexahydrofalconensin A (12) Hexahydrofalconensin A (**12**) (70 mg) was dissolved in MeOH (2 ml) and NaOMe (23 mg) was added to the solution. The mixture was refluxed for 1 h, the solvent was evaporated *in vacuo* and then 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 [hexane–acetone (5:1)] to give a ketodiol (**13**) (27 mg).

Compound **13**: White amorphous powder. EI-MS m/z (%): 242.1521 (M^+, 242.1519 for $C_{13}H_{22}O_4$, 31), 224 (M−H_2O, 14), 206 (M−2H_2O, 15), 199 (M−C_3H_7, 16), 181 (M−H_2O−C_3H_7, 30), 163 (M−2H_2O−C_3H_7, 12), 95 (70), 43 (C_3H_7, 100). UV λ_{max}^{MeOH} nm (log ε): 282 (2.21). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1718 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.91 [3H, t, J=7.0 Hz, 3′-H_3 (Me)], 1.25—1.52 (6H, m), 1.57 (3H, s, 7-Me), 1.85 (1H, dddd, J=11.6, 5.0, 2.4, 1.2 Hz, 8a-H), 2.26 [1H, dd, J=13.9, 1.8 Hz, 5-H (eq.)], 2.35 (1H, m, 4a-H), 2.44 (1H, d, J=2.4 Hz, 8-OH), 2.96 [1H, dd, J=13.9, 7.0 Hz, 5-H (ax.)], 3.30 (1H, m, 3-H), 3.50 [1H, dd, J=11.6, 2.4 Hz, 1-H (ax.)], 3.94 (1H, dd, J=11.6, 2.4 Hz, 8-H), 3.96 (1H, s, 7-OH), 4.36 [1H, br d, J=11.6 Hz, 1-H (eq.)]. CD (c=3.3×10^{-4}, MeOH) $\Delta\varepsilon^{20}$ (nm): +0.3 (286).

Hydrolysis of Hexahydrofalconensin A (12) Hexahydrofalconensin A (**12**) (50 mg) was dissolved in MeOH (3 ml) and NaOH (8 mg) in H_2O (2 ml) was added to the solution. The reaction mixture was warmed at 60 °C for 15 min, poured into ice-water 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 [hexane–acetone (5:1) and/or cyclohexane–AcOEt (10:9)] to give a ketodiol (**13**) (5 mg). This compound was identical with that obtained by methanolysis of **1**, based on comparisons of the ^{1}H-NMR, UV, IR, and CD spectra, and TLC behavior.

Synthesis of (*S*)- and (*R*)-MTPA Ester of the Ketodiol (13) Dicyclohexylcarbodiimide (30 mg), 4-dimethylaminopyridine (10 mg), and (*S*)- or (*R*)-MTPA (30 mg) were added to a solution of the ketodiol (**13**) (5 mg) in CH_2Cl_2 (4 ml). The reaction mixture was kept at 60 °C for 1.5 h, then poured into ice-water, and extracted with CH_2Cl_2. The extract was washed with 0.5 N HCl, saturated $NaHCO_3$, and water successively, and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by LPLC [hexane–acetone (9:1)] to afford the (*S*)- or (*R*)-MTPA ester of **13** [3 mg for (*S*), 4 mg for (*R*)].

(*S*)-MTPA Ester of **13**: White amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 0.89 [3H, t, J=7.0 Hz, 3′-H_3 (Me)], 1.10 [1H, m, 4-H (ax.)], 1.25—1.40 (4H, m), 1.37 (3H, s, 7-Me), 1.50—1.54 (1H, m), 2.00 (1H, br d, J=11.6 Hz, 8a-H), 2.32 [1H, br d, J=14.2 Hz, 5-H (eq.)], 2.38 (1H, m, 4a-H), 2.96 [1H, dd, J=14.2, 6.9 Hz, 5-H (ax.)], 3.27 (1H, m, 3-H), 3.37 [1H, dd, J=12.2, 2.4 Hz, 1-H (ax.)], 3.61 (3H, s, -OMe), 3.69 [1H, br d, J=12.2 Hz, 1-H (eq.)], 3.97 (1H, s, 7-OH), 5.63 (1H, d, J=11.6 Hz, 8-H), 7.42 (3H, m, aromatic-H), 7.67 (2H, m, aromatic-H).

(*R*)-MTPA Ester of **13**: White amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 0.89 [3H, t, J=7.0 Hz, 3′-H_3 (Me)], 1.09 [1H, m, 4-H (ax.)], 1.21—1.43 (4H, m), 1.24 (3H, s, 7-Me), 1.50—1.54 (1H, m), 2.08 (1H, br dd, J=11.6, 4.6 Hz, 8a-H), 2.32 [1H, dd, J=14.2, 1.7 Hz, 5-H (eq.)], 2.40 (1H, m, 4a-H), 2.95 [1H, dd, J=14.2, 6.9 Hz, 5-H (ax.)], 3.29 (1H, m, 3-H), 3.48 [1H, dd, J=12.2, 2.4 Hz, 1-H (ax.)], 3.59 (3H, s, -OMe), 3.84 (1H, s, 7-OH), 3.87 [1H, br d, J=12.2 Hz, 1-H (eq.)], 5.60 (1H, d, J=11.6 Hz, 8-H), 7.40 (3H, m, aromatic-H), 7.62 (2H, m, aromatic-H).

Methanolysis of Falconensins E (5) and F (6) Followed by Hydrolysis Falconensins E (**5**) (40 mg) and F (**6**) (41 mg) were each dissolved in MeOH (2 ml) and NaOMe (11.8 mg) was added to the solution. After refluxing for 20 min, the solvent was evaporated *in vacuo* and 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 hydrolyzed in a mixture of 10% NaOH (2 ml) and MeOH (1 ml) by refluxing for 12 h. After cooling, the reaction mixture was acidified with 4 N HCl and extracted with $CHCl_3$, and the solvent was evaporated *in vacuo*. After purification by LPLC (benzene), the residue was crystallized from hexane–CH_2Cl_2 to give 5-chloro-2,4-dimethoxy-6-methylbenzoic acid (**14**) (7 mg) from **5** and 2,4-dimethoxy-6-methylbenzoic acid (**15**) (6 mg) from **6**.

Compound **14**: Colorless needles, mp 210.5 °C (from MeOH). Beilstein test: positive (green). EI-MS m/z (%): 230 (M^+, 100), 232 (M+2, 36). IR ν_{max}^{KBr} cm^{-1}: 3050—2400, 1700 (COOH). UV λ_{max}^{MeOH} nm (log ε): 205 (3.96), 246 sh (3.02), 282 (2.86). ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, s, 6-Me), 3.87 (3H, s, 4-OMe), 3.95 (3H, s, 2-OMe), 6.74 (1H, s, 3-H).

Compound **15**: Colorless needles, mp 146 °C (from CCl_4). EI-MS m/z

(%): 196 (M^+, 100). IR ν_{max}^{KBr} cm^{-1}: 3050—2400, 1680 (COOH). UV λ_{max}^{MeOH} nm (log ε): 205 (3.98), 238 sh (3.19), 280 (2.71). ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, s, 6-Me), 3.84 (3H, s, 4-OMe), 3.97 (3H, s, 2-OMe), 6.40 (1H, d, J=1.8 Hz, 3-H), 6.46 (1H, d, J=1.8 Hz, 5-H).

Hydrogenation of Falconensins E (5) and F (6) with 10% Pd–C for 2 h Followed by Methanolysis The catalyst, 10% Pd–C (10 mg), was suspended in a solution of falconensins E (**5**) (94 mg) and/or F (**6**) (113 mg) in MeOH (6 ml) and the mixture was stirred at room temperature in a hydrogen atmosphere for 2 h. The catalyst was filtered off and the solvent was evaporated *in vacuo*. The residue was purified by LPLC with hexane–acetone (5 : 1). Each product was dissolved in MeOH (2 ml) and NaOMe (23 mg) was added. After refluxing for 1 h, the solvent was evaporated *in vacuo* and 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 [hexane–acetone (5 : 1)] to give a ketodiol (**10**) (14 mg from **5**; 12 mg from **6**), which was identical with the ketodiol derived from falconensin A (**1**) based on comparisons of the ^{1}H-NMR, UV, IR, and CD spectra and TLC behavior.

Acetylation of Falconensin F (6) Falconensin F (**6**) (16 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. The reaction mixture was poured into ice-water and extracted with ether, and the extract dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by LPLC with hexane–acetone (5 : 1) to afford *O*-acetylfalconensin F (**7**) (12 mg), which was identical with naturally occurring falconensin G based on comparisons of the ^{1}H-NMR, UV, IR, and CD spectra, and TLC behavior and the mixed melting point.

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