A New Azaphilone, Falconensin H, from *Emericella falconensis*

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A new azaphilone derivative, falconensin H (6), was isolated from mycelia of *Emericella falconensis*, together with previously reported falconensins A—D (1—4) and 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether (5). The structure of 6 was elucidated by spectroscopic investigation and chemical correlations. Among the compounds isolated, only 5 showed antibacterial activity.

Keywords *Emericella falconensis*; azaphilone; falconensin H; 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether; antibacterial activity

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] Recently we isolated four hydrogenated azaphilones, falconensins A (**1**), B(**2**), C(**3**) and D(**4**), from the dichloromethane extract of the mycelia of *E. falconensis*, strain NHL 2999(=ATCC 76117).[2] Further investigation on the antibacterial principle of this fungus against *Bacillus subtilis* COHN resulted in the isolation of 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether (**5**) along with a new compound designated as falconensin H (**6**). Among these compounds, **5**, which is a known constituent of *Emericella rugulosa* (THOM *et* RAPER) C. R. BENJAMIN[3] and *Hypocrea lactea* FRIES,[4] was the only one to show antibacterial activity. In this paper, we describe the structural determination of **6**.

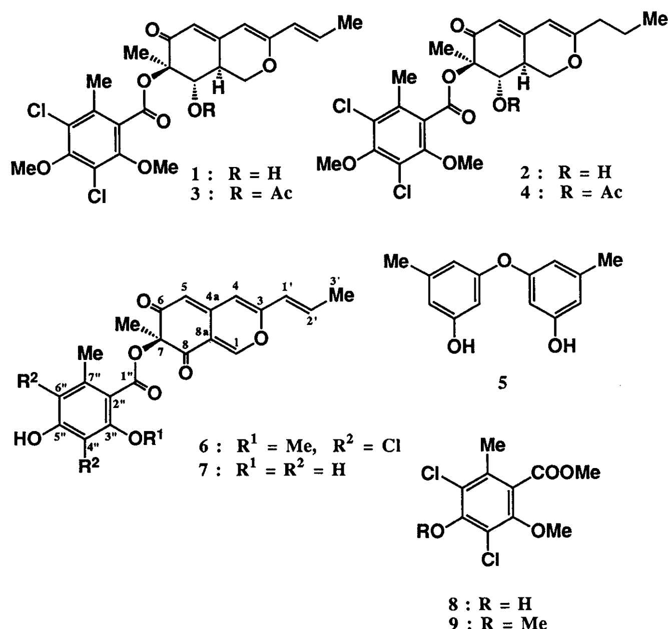
The molecular formula of falconensin H (**6**) was determined by high-resolution mass spectrometry as $C_{22}H_{18}Cl_2O_7$. The ^{13}C-NMR spectrum of **6** (Table I) showed the presence of three methyl carbons (δ 17.11, 18.58, 22.71), one methoxyl carbon (δ 62.60), one quaternary sp^3 carbon bearing an oxygen atom (δ 85.55), two conjugated ketone carbons (δ 191.83, 192.52), one ester carbonyl carbon (δ 165.22), five tertiary sp^2 carbons, and nine quaternary sp^2 carbons. The degree of the unsaturation suggests that **6** should be a tricyclic compound, like azaphilone. The presence of a 1-propenyl group was suggested by the ^{1}H-NMR signals at δ 6.01 (1H, dq), 6.58 (1H, dq), and 1.94 (3H, dd). The assignments of the ^{13}C-NMR signals were based on analysis of the ^{13}C–^{1}H shift correlation spectrum of **6**.

TABLE I. ^{1}H- and ^{13}C-NMR Chemical Shifts of Falconensin H (**6**) in $CDCl_3$

Carbon No.	δ_C (ppm)	δ_H (ppm)	Correlated protons with the carbon in the COLOC spectrum
1	153.64	7.94	
3	155.57[a]		
4	107.85	5.63	5-H
4a	142.77		1-H
5	108.52	6.12	4-H
6	192.52[b]		7-Me
7	85.55		5-H, 7-Me
7-Me	22.71	1.62	
8	191.83[b]		7-Me
8a	115.10		1-H, 4-H, 5-H
1′	122.43	6.01	3′-H
2′	135.55	6.58	
3′	18.58	1.94	
1″	165.22		
2″	117.86		3″-OMe, 7″-Me
3″	153.55		3″-OMe
3″-OMe	62.60	3.97	
4″	121.04		
5″	150.09[a]		
6″	117.36		7″-Me
7″	134.95		7″-Me
7″-Me	17.11	2.49	

a, *b*) Assignments may be interchanged.

The structure of the azaphilone part in falconensin H (**6**) was determined by analysis of the ^{13}C–^{1}H correlation *via* long-range coupling (COLOC) spectrum (Table I). The proton signal at δ 7.94 in **6** could reasonably be assigned to the 1-H proton of the azaphilone moiety. This proton was observed at δ 8.25 in mitorubrin (**7**), a constituent of *Penicillium rubrum* STOLL.[5] From the above results, the azaphilone part of **6** was determined. The other part was identified by methanolysis of **6**, yielding a methyl benzoate derivative (**8**), which was identical with methyl 3,5-dichloro-4-hydroxy-2-methoxy-6-methylbenzoate.[6] On methylation, **8** gave methyl 3,5-dichloro-2,4-dimethoxy-6-methylbenzo-



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ate (**9**), identical with the compound derived from **1** by methanolysis.[2] The relative structure of falconensin H was thus determined as **6**.

The absolute configuration at C-7 of azaphilones so far known has been firmly established from the optical rotations and circular dichroism (CD) curves.[7,8] The sign of the Cotton effect at the longest wavelength depends on the stereochemistry at the C-7 position [(+), α-methyl; (−), β-methyl]. The CD of 6 ($\Delta\varepsilon_{368}$ +2.5) clearly showed (*S*)-configuration at C-7. The absolute structure of falconensin H was consequently concluded to be as shown in **6**.

The mycelial extract of *E. falconensis* exhibited antibacterial activity against *B. subtilis* as mentioned above. However, the azaphilone derivatives isolated, falconensins A—H, had no activity up to 200 μg/disc. Only 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether (**5**) had antibacterial activity, giving inhibition circles of 14 and 12 mm at the concentrations of 100 and 50 μg/disc, respectively.

Experimental

General Procedures Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotation was measured with a JASCO DIP-181 spectrometer. Electron impact-mass spectra (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. The coupling patterns are indicated as follows: singlet = s, doublet = d, triplet = t, and quartet = q, 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. × 150 or 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 under UV light.

Isolation of Metabolites from *Emericella falconensis* *E. falconensis*, strain NHL 2999, was cultivated in Czapek medium supplemented with 0.2% yeast extract (30 l) in 120 Roux flasks at 25 °C for 21 d. The dried mycelia (227 g) were 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 chromatographed on silica gel (400g, i.d. 3 × 20 cm) with benzene–AcOEt (20 : 1) to give the fraction containing falconensins A—D, and with benzene–AcOEt (2 : 1) followed by purification by repeated LPLC with hexane–acetone (5 : 1) to afford falconensin H (**6**) (6 mg). The former fraction was further subjected, after separation of falconensins A—D (**1**—**4**),[2] by repeated LPLC with hexane–acetone (4 : 1) to afford 3,3′-dihydroxy-5,5′-dimethyldiphenyl ether (**5**) (230 mg).

Compound **5**: Colorless viscous oil. EI-MS *m/z* (%): 230.0944 (M$^+$, 230.0943 for $C_{14}H_{14}O_3$, 100). UV λ_{max}^{MeOH} nm (log ε): 225 (4.26), 276 (3.61), 282 (3.60). ^{1}H-NMR ($CDCl_3$) δ: 2.22 (6H, s, 5-Me, 5′-Me), 5.90 (2H, s, 3-OH, 3′-OH), 6.28 (2H, t, *J* = 2.2 Hz, 2-H, 2′-H), 6.39 (4H, d, *J* = 2.2 Hz, 4-H, 6-H, 4′-H, 6′-H).

Falconensin H (**6**): Yellow crystalline powder, mp 63 °C (from AcOEt), $[\alpha]_D^{20}$ +13.7° (*c* = 0.19, MeOH). EI-MS *m/z* (%): 464.0420 (M$^+$, 464.0428 for $C_{22}H_{18}{}^{35}Cl_2O_7$, 14), 466.0397 (M + 2, 466.0398 for $C_{22}H_{18}{}^{35}Cl{}^{37}ClO_7$, 10), 468.0367 (M + 4, 468.0368 for $C_{22}H_{18}{}^{37}Cl_2O_7$, 3), 250 (5), 233 ($C_9H_7Cl_2O_3$, 100), 235 (63), 237 (10). UV λ_{max}^{MeOH} nm (log ε): 248 (4.27), 282 (4.10), 294 (4.08), 346 (4.26). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1715 (–COO–), 1650 (cojugated C = O). ^{1}H-NMR ($CDCl_3$) δ: 1.62 (3H, s, 7-Me), 1.94 (3H, dd, *J* = 7.1, 1.5 Hz, 3′-Me), 2.49 (3H, s, 7″-Me), 3.97 (3H, s, 3″-OMe), 5.63 (1H, s, 4-H), 6.01 (1H, dq, *J* = 15.6, 1.5 Hz, 1′-H), 6.12 (1H, s, 5-H), 6.58 (2H, dq, *J* = 15.6, 7.1 Hz, 2′-H), 7.94 (1H, s, 1-H). CD (*c* = 1.8 × 10^{-4}, MeOH) $\Delta\varepsilon^{20}$: +2.4 (241), +2.4 (247), −1.3 (270), +0.6 (333), +2.5 (368).

Methanolysis of Falconensin H (6) Falconensin H (**6**) (14 mg) was dissolved in MeOH (1 ml) and NaOMe (5.9 mg) was added to the solution. The whole was refluxed for 20 min, then the solvent was evaporated off *in vacuo* and the residue was acidified with dilute HCl and extracted with $CHCl_3$. The extract was drid over Na_2SO_4 and the solvent was removed by evaporation. The residue was purified by LPLC to give methyl 3,5-dichloro-4-hydroxy-2-methoxy-6-methylbenzoate (**8**) (2 mg).

Compound **8**: Colorless crystalline powder, mp 81 °C. Beilstein test, positive (green). EI-MS *m/z*: 264 (M$^+$, 100), 266 (M + 2, 71), 268 (M + 4, 12). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (–COO–). ^{1}H-NMR ($CDCl_3$) δ: 2.31 (3H, s, 6-Me), 3.80 (3H, s, –COOMe), 3.88 (3H, s, 2-OMe), 6.30 (1H, s, 4-OH).

Methylation of Compound 8 The Et_2O solution of CH_2N_2 (2 ml) was added to a solution of **8** (10 mg) in Et_2O (2 ml) and the mixture was kept overnight at room temperature. After removal of the ether by evaporation, the residue was purified by LPLC (benzene) to obtain methyl 3,5-dichloro-2,4-dimethoxy-6-methylbenzoate (**9**) (7 mg), which was shown to be identical with an authentic sample by comparison of the IR, UV and ^{1}H-NMR spectra and the TLC behavior.

Determination of Antibacterial Activity The antibacterial activity was detemined by means of the paper disc assay with *B. subtilis* as the test organism. The assay plates were prepared using Antibiotic medium 3 (Difco) (0.35 g) and agar (0.40 g) in distilled water (20 ml). Bacteria cultivated in the above medium for 2 d were suspended in water and spread over the surface of the assay plates with cotton. The metabolites dissolved in Me_2CO or MeOH were charged onto paper discs (8 mm diameter) in the amounts mentioned in the text, and the discs were dried and placed on the assay plates. Zones of inhibition (mm in diameter) were recorded after a 24 h incubation at 37 °C.

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