Notes

Dehydroxychlorofusarielin B, an Antibacterial Polyoxygenated Decalin Derivative from the Marine-Derived Fungus *Aspergillus* sp.

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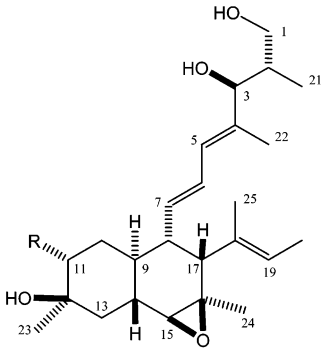
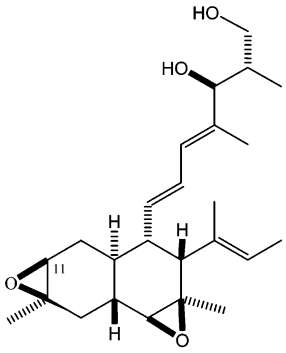
Dehydroxychlorofusarielin B (**1**), a new antibacterial polyoxygenated decalin derivative, and the previously described fusarielins A (**2**) and B (**3**) have been isolated from the broth of a marine isolate of the fungus *Aspergillus*. The structure and absolute stereochemistry of the new compound was determined on the basis of the physicochemical data and X-ray diffraction. Compounds **1**−**3** exhibited a mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The MIC values for each strain were as follows: compounds **1** and **3**, 62.5 μg/mL for all strains; compound **2**, 32.5 μg/mL for *S. aureus* and methicillin-resistant *S. aureus* and 62.5 μg/mL for multidrug-resistant *S. aureus*.

Multidrug-resistant strains of many clinically important pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Staphylococcus aureus* (MDRSA), are posing a worldwide health problem.[2,3] Thus, there is an urgent need to discover new agents to treat patients infected with methicillin-resistant and multidrug-resistant bacteria. For our screening aimed at identifying antimicrobial compounds of microbial origin,[4] we investigated the antibacterial activity of marine-derived fungal extracts against MRSA and MDRSA, and we successively isolated antibacterial polyoxygenated decalin derivatives, dehydroxychlorofusarielin B (**1**) and fusarielins A (**2**) and B (**3**),[5] from the marine-derived fungus *Aspergillus* sp. We report here on the isolation and structural elucidation of these metabolites.



dehydroxychlorofusarielin B (**1**): R = Cl

fusarielin B (**3**): R = OH



fusarielin A (**2**)

Dehydroxychlorofusarielin B (**1**) was obtained in the form of colorless crystalline aggregates from *n*-hexane−acetone. It showed an isotopic cluster at *m*/*z* 438 and 440 with the ratio of 3:1 in the EIMS, suggesting the presence of a chlorine atom. A molecular formula of $C_{25}H_{39}ClO_4$, which gave six degrees of unsaturation, was established by HREIMS and ^{13}C NMR methods. The IR band at 3434 cm^{-1} indicated the presence of a hydroxyl functionality in **1**. The UV spectrum of **1** revealed the presence of a conjugated diene chromophore [272 nm (log ϵ = 2.68)]. In the ^1H NMR spectrum, three protons were exchanged by D_2O, suggesting that **1** had three hydroxyl protons [4.23 (1H, dd, J = 5.5, 5.0 Hz, 1-OH); 4.61 (1H, d, J = 3.8 Hz, 3-OH); 4.83 (1H, s, 12-OH)]. Detailed analyses of the ^1H and ^{13}C NMR spectra of **1**, including DEPT, COSY, HMQC, and HMBC experiments, revealed signals ascribable not only to 5,7-dihydroxyl-4,6-dimethyl-1,3-heptadienyl and 1-methyl-1-propenyl groups but also to an octasubstituted decalin moiety having a chloro, a hydroxyl, two methyls, and an oxirane group (Table 1, Figure 1). The connection and position of the functional groups in **1** were achieved on the basis of COSY and HMBC correlations. Key COSY and HMBC correlations showed the connections of C7−C8 and C17−C18 bonds as well as the positions of 11-chloro, 12-hydroxyl, 12-methyl, 15,16-epoxy, and 16-methyl groups (Figure 1). These spectroscopic features revealed that compound **1** had the general structural features of fusarielin B (**3**).[5] The NMR data of both of these compounds showed similar patterns, except for the loss of one hydroxyl proton, the downfield shift of H-11 [3.98 (1H, br s); $\Delta\delta$ = 0.7 ppm], and upfield shift of C-11 [66.1 (CH); $\Delta\delta$ = 6.7 ppm] in **1**. Thus, compound **1** was characterized as an 11-dehydroxychloro derivative of fusarielin B, and direct comparison of NMR data of **1** with those of **3** provided additional support in justifying the gross structure shown for **1**.

The relative stereochemistry of **1** was determined by a direct comparison of the NMR data of **1** with those of **3**[5] (Table 1), except for the stereochemistry at C-11. The relative stereochemistry at C-11 had yet to be assigned because H-11 [3.98 (1H, br s)] showed neither the coupling constants with H_2-10 nor NOE correlations with any protons of asymmetric carbons. The stereochemistry at C-8 and C-17 was further supported by an observation of NOE correlations between H-8 and H-17, which showed that they oriented on the same face of the molecule. To establish the stereochemistry of dehydroxychlorofusarielin B (**1**), an X-ray diffraction analysis of **1** was performed (see Figure S3 in the Supporting Information). The crystal packing was stabilized by strong intramolecular and intermolecular hydrogen bonds among the asymmetric molecules such as O1−H1···O1′, O2−H2···O3′, O1′−H1′···O2′, and O3′−

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Table 1. NMR Spectroscopic Data (400 MHz, DMSO-d_6, 50 °C) for Dehydroxychlorofusarielin B (**1**)

position	δ_C, mult.	δ_H (J in Hz)
1	63.7, CH_2	3.31, ddd (10.5, 6.0, 5.5)
		3.54, ddd (10.5, 5.0, 4.0)
2	38.3, CH	1.61, m[a]
3	78.7, CH	3.65, dd (8.5, 3.8)
4	137.6, qC	
5	125.1, CH	5.83, d (10.8)
6	126.7, CH	6.19, dd (15.0, 10.8)
7	134.2, CH	5.12, dd (15.0, 10.2)
8	42.5, CH	2.22, ddd (11.0, 10.2, 5.0)
9	29.7, CH	1.83, dddd (12.3, 11.0, 11.0, 4.3)
10	34.4, CH_2	1.52, 1.64, m[a]
11	66.1, CH	3.98 br s
12	70.8, qC	
13	37.8, CH_2	1.59, m[a]
14	35.9, CH	1.90, ddd (12.3, 11.0, 3.2)
15	62.7, CH	2.69, s
16	60.2, qC	
17	53.2, CH	2.54, d (5.0)
18	133.0, qC	
19	124.4, CH	5.24, q (6.5)
20	13.1, CH_3	1.60, d (6.5)
21	13.7, CH_3	0.68, d (7.0)
22	11.4, CH_3	1.64, s[b]
23	28.3, CH_3	1.25, s
24	21.7, CH_3	1.14, s
25	18.0, CH_3	1.62, s[b]
1-OH		4.23, dd (5.5, 5.0)
3-OH		4.61, d (3.8)
12-OH		4.83, s

[a] Signal partially overlapped. [b]Interchangeable assignments.

H3′···O3 (see Table S1 and Figure S4 in the Supporting Information). The absolute configuration for **1** was also unambiguously determined by refining the Flack parameter.[6]

The known compounds **2** and **3** were identified by spectroscopic analysis (^{1}H and ^{13}C NMR, LREIMS, and $[\alpha]_D$) and comparison to literature data.[5,7]

Antifungal antibiotic fusarielins A−D were isolated from *Fusarium* sp.,[5,7] and their derivatives, ICM0301s, of angiogenesis inhibitory activity were isolated from *Aspergillus* sp.[8] Compounds **1**−**3** exhibited a mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The MIC values for each strain were as follows: compounds **1** and **3**, 62.5 μg/mL for all strains; compound **2**, 32.5 μg/mL for *S. aureus* and methicillin-resistant *S. aureus* and 62.5 μg/mL for multidrug-resistant *S. aureus*.

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer model 341 polarimeter. UV/visible spectra were measured on a Hitachi U-2001 UV/vis spectrometer. IR spectra were recorded on a Bruker FT-IR model IFS-88 spectrometer. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained at 50 °C on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks [DMSO-d_6: ^{1}H (δ 2.50) and ^{13}C (δ 39.5)] as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. Single-crystal X-ray measurements were performed on a Bruker SMART CCD diffractometer.

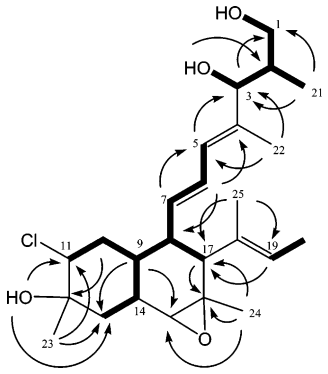

Figure 1. Structure of **1** elucidated by ^{1}H−^{1}H COSY (—) and HMBC (→) correlations.

Fungal Isolation and Culture. The fungal strain was isolated from the surface of the marine brown alga *Sargassum horneri* collected at Gadeok Island, Busan, Korea, in 2001, and identified as an *Aspergillus* sp. (family Trichocomaceae) on the basis of fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea, similarity index of 0.87). A voucher specimen is deposited at Pukyong National University with the code MFB024. The fungus was cultured (20 L) for 3 weeks (static) at 29 °C in SWS medium consisting of soytone (0.1%), soluble starch (1.0%), and seawater (100%).

Extraction and Isolation. The mycelium and broth were separated by filtration. The filtered broth was extracted with EtOAc to afford broth extract (1.7 g), which was subjected to Si gel flash chromatography. Elution was performed with *n*-hexane−EtOAc (stepwise, 0−100% EtOAc) to yield four fractions. Fractions 3 and 4 were separated by medium-pressure liquid chromatography (MPLC) (ODS) using a H_2O−MeOH gradient elution to afford crude compounds **1**, **2**, and **3**, respectively. These were further purified by HPLC (YMC, ODS-A) utilizing a 30 min gradient program of 50% to 100% MeOH in H_2O to furnish **1** (10.5 mg), **2** (4.8 mg), and **3** (8.4 mg), respectively.

Dehydroxychlorofusarielin B (1): colorless, crystalline aggregates from *n*-hexane−acetone: $[\alpha]^{20}_D$ −125 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (2.68) nm; IR (KBr) ν_{max} 3434, 2961, 2928, 2870, 1638, 1458, 1379, 1090, 1028, 1011, 968, 756 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 440 $[M]^+$ (0.3), 438 $[M]^+$ (1.0), 420 [M − H_2O] (11), 404 (0.5), 389 (4), 379 (17), 349 (6), 325 (6), 307 (15), 295 (21), 277 (15), 259 (14), 241 (12), 235 (15), 211 (28), 197 (20), 185 (27), 173 (32), 157 (45), 147 (39); HREIMS *m*/*z* 438.2537 (calcd for $C_{25}H_{39}{}^{35}ClO_4$, 438.2537), 440.2500 (calcd for $C_{25}H_{39}{}^{37}ClO_4$, 440.2519).

Fusarielins A (2) and B (3): white powder; spectroscopic data virtually identical to those reported in the literature.[5]

Antibacterial Assay. The *in vitro* antibacterial activity of the fermentation broth and purified samples were evaluated by a conventional 2-fold serial dilution method using *S. aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus* as indicator strains. A 5 mL suspension containing 10^5 cells per milliliter was used as inoculum of the test organism. The MIC values were determined after the inoculation for 18 h at 37 °C.[9]

X-ray crystallographic analysis of compound 1: $C_{25}H_{39}ClO_4 \cdot 0.75(H_2O)$, M_w = 452.52, T = 173(2) K, λ = 0.71073 Å, orthorhombic, $P2_12_12_1$, a = 11.0682(6) Å, b = 17.380(1) Å, c = 29.355(2) Å, V = 5646.9(6) Å^3, Z = 8, d_{calcd} = 1.065 Mg m^{-3}, $F(000)$ = 1964. The final R_1 and wR_2 values with 12 269 Friedel pair reflections ($[I > 2\sigma(I)]$) were 0.1050 and 0.2329, respectively. Absolute structure parameter χ = −0.02(15).[6] Crystallographic data have been deposited with the Cambridge Crystallographic Data Center (deposit No. CCDC 625789). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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Supporting Information Available: Experimental refinement, ^{1}H and ^{13}C NMR spectra (at 50 °C), an ORTEP III drawing, hydrogen-bonding geometry and scheme, and tabulated bond lengths and angles in the crystal structure of dehydroxychlorofusarielin B (**1**). These materials are available free of charge via the Internet at http://pubs.ac.org.

References and Notes

(1) This author contributed to a part of this work as an exchange student from College of Chemistry & Chemical Engineering, Ocean University of China, Qingdao 266003, People's Republic of China.
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