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Chrodrimanins I and J from the Antarctic Moss-Derived Fungus *Penicillium funiculosum* GWT2-24

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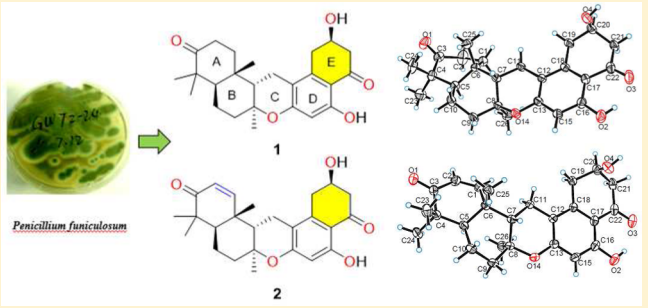
Supporting Information

ABSTRACT: Two new meroterpenoids, named chrodrimanins I and J (**1** and **2**), together with five known biosynthetically related chrodrimanins (**3**−**7**), were isolated from the culture of the Antarctic moss-derived fungus *Penicillium funiculosum* GWT2-24. Distinguished from all of the reported chrodrimanins, compounds **1** and **2** possess a unique cyclohexanone (E ring) instead of a δ-lactone ring. These structures including the absolute configurations were established on the basis of MS, NMR, and X-ray crystallographic analysis. Compounds **1**−**7** showed no cytotoxic or antibacterial activities, while the known compounds **3**, **5**, and **6** exhibited inhibitory activities against influenza virus A (H1N1), with IC_{50} values ranged from 21 to 57 μM.

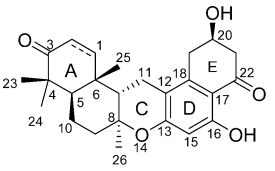


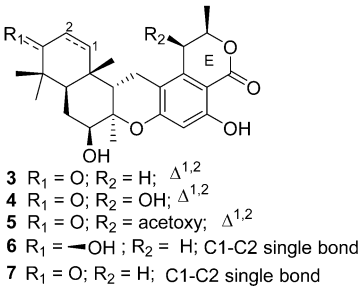
Chrodrimanins represent a family of meroterpenoids consisting of a C10 polyketide unit and a sesquiterpene moiety.[1] Following the first isolation from a *Penicillium* strain,[2] only 12 analogues, chrodrimanins A−H (chrodrimanins B and H have also been named as thailandolides A and B[1,3]) and pentacecilides A−D,[4,5] have been discovered from a range of fungi including *Talaromyces* sp.[1,6] and *Aspergillus* sp.[7] in addition to *Penicillium* sp. All of the reported chrodrimanins/pentacecilides share an interesting 6/6/6/6/6 pentacyclic ring system including a hydrogenated pyran (C ring) and a δ-lactone (E ring). These compounds have displayed various biological activities such as insecticidal activity[1,2] and lipid droplet formation inhibition.[5,8]

In our continuing exploration of secondary metabolites produced by Antarctic-derived fungi,[9] *Penicillium funiculosum* GWT2-24, isolated from moss collected near the China Great Wall Station, was selected for further research due to the interesting HPLC-UV profiles. From the EtOAc extract, two new meroterpenoids, named chrodrimanins I and J (**1** and **2**), together with five known analogues, namely, chrodrimanins A and B (**3** and **4**),[2,7] E and F (**5** and **6**), and H (**7**),[1] were isolated. Herein, we report the details of their isolation, structure elucidation, and bioactivity screening.

The fungal strain *P. funiculosum* GWT2-24 (30 L) was fermented with shaking at 28 °C for 8 days. The EtOAc extracts of the broth and mycelia were fractionated by repeated chromatography to yield the new compounds **1** (5.6 mg) and **2** (3.0 mg).

Chrodrimanin I (**1**) was obtained as colorless needles, and its molecular formula was established as $C_{25}H_{32}O_5$ by the HRESIMS ion at m/z 413.2331 $[M + H]^+$, indicating 10 degrees of unsaturation. The ^1H and ^{13}C NMR spectroscopic





data (Table 1) revealed the presence of 10 nonprotonated carbons including two ketones (δ_C 218.0, 202.6) and five aromatic ones, four methines with one oxygenated, seven sp^3 methylenes, and four methyls. The planar structure was constructed on the basis of 2D NMR relationships. The COSY correlations between H-1 and H-2, between H-5 and H-10, between H-10 and H-9, and between H-7 and H-11 indicated the three spin systems in the sesquiterpene moiety, which were extended to form the A and B rings evidenced by the HMBC correlations from H-2 to C-4 and C-6, from H-5 to

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Table 1. ^{1}H (600 MHz) and ^{13}C NMR (125 MHz) Data of 1 and 2 in DMSO-d_6

	1		2	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1a	33.8, CH_2	2.85, m	157.8, CH	7.49, d (9.9)
1b		2.22, m		
2a	31.2, CH_2	2.05, m	127.1, CH	5.89, d (9.9)
2b		1.55, m		
3	218.6, C		204.1, C	
4	46.7, C		44.7, C	
5	46.7, CH	1.94, m	44.3, CH	2.02, m
6	35.3, C		38.9, C	
7	44.7, CH	1.76, dd (13.2, 4.4)	48.0, CH	1.84, dd (14.0, 4.4)
8	78.9, C		78.6, C	
9a	38.6, CH_2	2.05, m	35.8, CH_2	2.02, m
9b		1.94, m		
10a	18.1, CH_2	1.55, m	17.1, CH_2	1.75, m
10b				1.69, m
11a	20.0, CH_2	2.54, dd (4.4, 16.5)	22.3, CH_2	2.84, dd (4.4, 15.4)
11b		2.42, dd (13.2, 16.5)		2.48, dd (14.0, 15.4)
12	112.8, C		113.5, C	
13	160.6, C		160.4, C	
15	101.8, CH	6.05, s	102.1, CH	6.09, s
16	162.4, C		162.5, C	
17	111.6, C		111.7, C	
18	143.5, C		143.8, C	
19a	35.1, CH_2	2.95, dd (16.5, 4.4)	35.2, CH_2	3.03, dd (16.5, 4.4)
19b		2.82, dd (16.5, 6.6)		2.88, dd (16.5, 7.7)
20	65.0, CH	4.19, m	65.0, CH	4.20, m
21a	46.8, CH_2	2.85, m	47.0, CH_2	2.81, dd (16.5, 3.3)
21b		2.59, dd (16.4, 8.2)		2.61, dd (16.5, 8.8)
22	203.1, C		203.2, C	
23	20.2, CH_3	0.96, s	21.5, CH_3	1.04, s
24	28.8, CH_3	1.04, s	27.8, CH_3	1.07, s
25	22.9, CH_3	0.88, s	28.1, CH_3	1.25, s
26	25.3, CH_3	1.31, s	23.7, CH_3	1.18, s
16-OH		12.61, s		12.63, s
20-OH		5.18, d (3.3)		5.22, d (4.4)

C-4 and C-6, from H-25 to C-1, C-6, and C-7, and from H-26 to C-7, C-8, and C-9. The hydrogenated naphthalene ring (D and E rings) was indicated by the COSY correlations between H-19 and H-20 and between H-20 and H-21 and the HMBC correlations from H-15 to C-13, C-16, and C-17, from H-19 to C-17, C-18, and C-20, and from H-21 to C-20 and C-22, together with their chemical shifts. The COSY correlations between the exchangeable OH-20 and H-20 and the HMBC correlations from OH-16 to C-15 and C-16 located the two hydroxy groups on C-20 and C-16, respectively. The additional HMBC correlations from H-11 to C-12 indicated the connection of the sesquiterpene unit and the naphthalene group by C-11. With nine unsaturation degrees accounted for and one oxygen atom remaining, the gross structure was established by forming a pyran ring through the connection of C-8 and C-13 via O-14. The chemical shifts of the sesquiterpene moiety agreed with the known compound chrodrimanin C.[6] The relative configurations of A and B rings in **1** were determined as 5*R**, 6*S**, 7*S**, 8*R** on the basis of NOESY cross-peaks between H-5 and H_3-26 and between H_3-25 and H-7 (Figure 2). Finally, the absolute configuration of **1** was determined as 5*R*, 6*S*, 7*S*, 8*S*, and 20*R* by X-ray crystallographic analysis (Figure 3) using Cu Kα radiation.

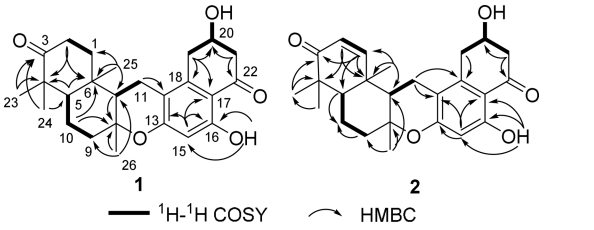

Figure 1. Key COSY and HMBC correlations of **1** and **2**.

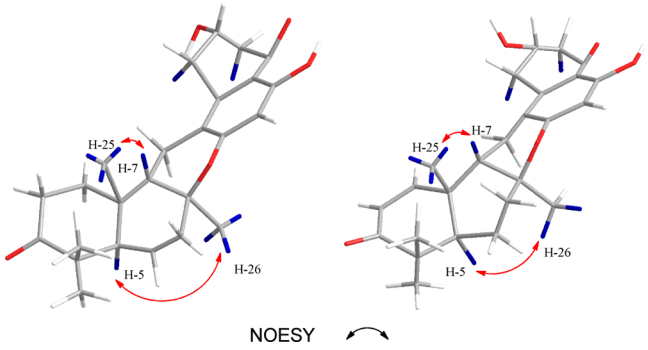

Figure 2. Key NOESY correlations of **1** and **2**.

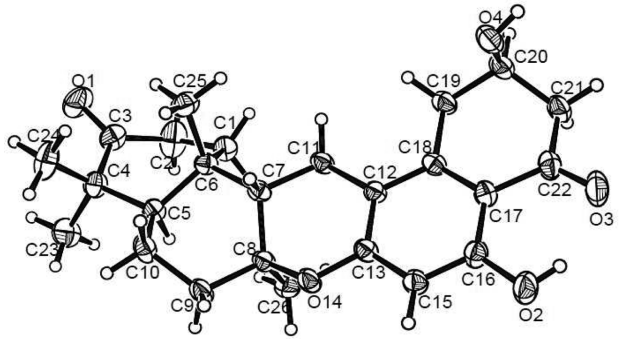

Figure 3. X-ray ORTEP diagram of **1**.

Compound **2** was also isolated as colorless needle crystals. The molecular formula of **2** was established as $C_{25}H_{30}O_5$ by HRESIMS. The ^{1}H and ^{13}C NMR spectroscopic features of **2** (Table 1) were similar to those of **1** with the exchange of the single bond between C-1 and C-2 in **1** for a double bond (δ_H 7.49, δ_C 157.2, CH-1; δ_H 5.89, δ_C 126.5, CH-2) in **2**, which was further supported by the 2D NMR data (Figure 1). The relative configurations of the sesquiterpene moiety in **2** were also established to be the same as **1** by the NOESY spectrum (Figure 2). The absolute configuration of **2** was also determined as 5*R*, 6*S*, 7*S*, 8*S*, and 20*R* by X-ray crystallographic analysis (Figure 4) with a Flack parameter of 0.00, and therefore **2** was named chrodrimanin J.

All compounds lacked cytotoxic activity (IC_{50} > 50 μM) on the four tested cell lines, K562, HL-60, HeLa, and A-549 (adriamycin as positive control),[10,11] and antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* (MIC > 600 μg/mL). The antiviral activities against influenza A virus

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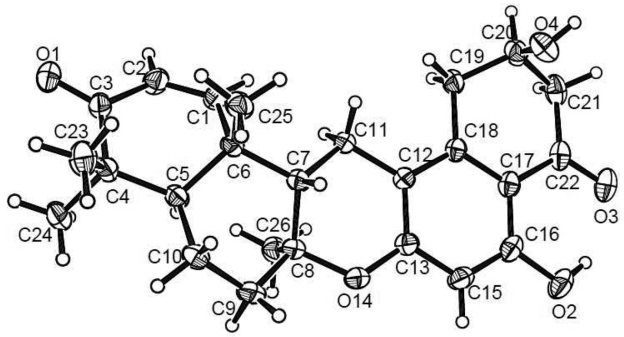

Figure 4. X-ray ORTEP diagram of **2**.

(H1N1)[12] of compounds **1**−**7** were also screened for the first time. Compounds **3**, **5**, and **6** displayed activity with IC_{50} values of 21, 55, and 57 μM, respectively, while the other compounds were inactive (IC_{50} > 200 μM) (ribavirin as positive control, IC_{50} = 115 μM).

The chrodrimanins have been proposed to form by an initial alkylation of 6-hydroxymellein with farnesyl diphosphate.[1] Unlike the known chrodrimanins, compounds **1** and **2** may originate from scytalone, an important intermediate of the fungal pigment melanin. It is notable that scytalone[13] and 6-hydroxymellein[14] are biosynthesized by two types of PKS, fungal nonreducing and partially reducing iterative polyketide synthases (NR-PKS and PR-PKS), respectively. Interestingly, in *P. funiculosum* GWT2-24, an NR-PKS and a PR-PKS both seem to be involved in the biosynthesis of chrodrimanins with cyclohexanone (**1** and **2**) and δ-lactone E rings (**3**−**7**), which helps to extend the structural diversity of fungal meroterpenoids.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting point was determined on an RY-1 micromelting point apparatus and is uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Waters 2487 absorbance detector. ECD spectra were recorded on a JASCO J-815 spectropolarimeter, using MeOH as solvent. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer as KBr disks. ^{1}H and ^{13}C NMR, DEPT, and 2D NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as an internal standard. ESIMS spectra were measured on a Micromass Q-TOF Ultima Global GAA076 LC mass spectrometer. HRESIMS spectra were measured on a Micromass EI-4000 (Autospec-Ultima-TOF). X-ray crystal data were measured on an Agilent Gemini Ultra diffractometer (Cu Kα radiation). Semipreparative HPLC was performed using an ODS column [YMC-pack ODS-A, 10 × 250 mm, 5 μm, 3 mL/min]. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF254 (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (GE Healthcare), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory).

Fungal Material. The fungal strain *Penicillium funiculosum* GWT2-24 was isolated from an inner part of moss collected around the China Great Wall Station in Antarctica and was identified by the ITS sequence with GenBank accession number JQ670957. A voucher specimen is deposited in our laboratory at −20 °C. The working strain was prepared on potato dextrose agar slants and stored at 4 °C.

Fermentation and Extraction. The fungus GWT2-24 was cultured under shaking conditions at 28 °C for 8 days in 500 mL Erlenmeyer flasks containing 150 mL of liquid culture medium, composed of glucose (1%), maltose (2%), mannitol (2%), monosodium glutamate (1%), KH_2PO_4 (0.05%), $MgSO_4 \cdot 7H_2O$ (0.03%), and yeast extract (0.3%) after adjusting its pH to 6.5. The fermented whole broth (30 L) was filtered through cheesecloth to separate the filtrate from the mycelia. The filtrate was extracted three times with an equivalent volume of EtOAc, which was concentrated under reduced pressure. The mycelia were extracted three times with acetone, followed by concentration under reduced pressure to afford an aqueous solution, which was further extracted three times with an equivalent volume of EtOAc. Both EtOAc solutions of broth and mycelia were combined and concentrated under reduced pressure to give an extract (15.6 g).

Purification. The extract was subjected to a VLC fractionation in an open column using silica as the solid phase and a gradient solvent system with petroleum ether−acetone of 10:1, 5:1, and 1:1, resulting in three VLC fractions (Fr.1−Fr.3). Fr.1 was subjected to a Sephadex LH-20 column eluting with CH_2Cl_2−MeOH (1:1, v/v) to give six subfractions (Fr.1.1−Fr.1.6). Fr.1.1 was fractionated on a silica column eluting with petroleum ether−acetone (15:1, v/v) and finally purified by semipreparative HPLC (MeOH−H_2O, 60:40) to afford compounds **3** (29.0 mg), **5** (31.5 mg), and **7** (9.0 mg). Fr.2 was separated into five subfractions, Fr.2.1−Fr.2.5, by Sephadex LH-20 column chromatography eluting with CH_2Cl_2−MeOH (1:1, v/v). Subfraction Fr.2.1 was prepared by semipreparative HPLC (ODS; 5 μm, 250 × 10 mm; MeOH−H_2O, 65:35, v/v; 3 mL/min) to give compounds **1** (5.6 mg), **2** (3.0 mg), and **6** (20 mg). Compound **4** (6.5 mg) was purified from Fr.3 using a similar procedure to that for Fr.2.1.

*Chrodrimanin I (**1**):* colorless needles; mp 238 °C; $[\alpha]^{26}_D$ −26 (*c* 0.1, $CHCl_3$); ECD (1.0 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 211 (−5.51), 233 (+3.71), 283 (−2.19), 309 (+0.96) nm; UV (MeOH) λ_{max} (log ε) 212 (0.90), 291 (1.07) nm; IR (KBr) ν_{max} 3405, 2957, 1668, 1631, 1478, 1382, 1306, 1263, 1163, 1123, 820 cm^{-1}; ^{1}H and ^{13}C NMR, Table 1; HRESIMS *m*/*z* 413.2331 $[M + H]^+$ (calcd for $C_{25}H_{33}O_5$ 413.2323).

*Crystal data for **1**:* monoclinic, $C_{25}H_{32}O_5$, space group $P2_1$ with *a* = 8.7283(2) Å, *b* = 25.8978(4) Å, *c* = 10.1531(2) Å, $\alpha = \gamma = 90°$, β = 109.86, *V* = 2158.55(7) $Å^3$, *Z* = 4, *T* = 291(2) K, μ(Cu Kα) = 0.702 mm^{-1}, D_c = 1.269 g/mm^3, and *F*(000) = 888.0. Crystal size: 0.39 × 0.32 × 0.31 mm^3. Independent reflections: 7914 with R_{int} = 0.0234. The final agreement factors are R_1 = 0.0337 and wR_2 = 0.0922 [$I \geq 2\sigma(I)$]. Flack parameter = 0.01(11).

*Chrodrimanin J (**2**):* colorless needles; mp 246 °C; $[\alpha]^{26}_D$ −24 (*c* 0.1, $CHCl_3$); ECD (1.0 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 211 (−3.51), 283 (−5.36), 309 (+2.87) nm; UV (MeOH) λ_{max} (log ε) 216 (1.50), 289 (1.45) nm; IR (KBr) ν_{max} 3410, 2954, 1658, 1631, 1477, 1382, 1308, 1263, 1162, 1123, 821 cm^{-1}; ^{1}H and ^{13}C NMR, Table 1; HRESIMS *m*/*z* 411.2165 $[M + H]^+$ (calcd for $C_{25}H_{31}O_5$ 411.2166).

*Crystal data for **2**:* monoclinic, $C_{25}H_{30}O_5$, space group $P2_1$ with *a* = 8.13420(10) Å, *b* = 18.9651(2) Å, *c* = 15.10890(10) Å, $\alpha = \gamma = 90°$, β = 102.4910(10)°, *V* = 2275.62(4) $Å^3$, *Z* = 4, *T* = 290(2) K, μ(Cu Kα) = 0.735 mm^{-1}, D_c = 1.292 g/mm^3, and *F*(000) = 952.0. Crystal size: 0.320 × 0.300 × 0.220 mm^3. Independent reflections: 7611 with R_{int} = 0.0157. The final agreement factors are R_1 = 0.0320 and wR_2 = 0.0887 [$I \geq 2\sigma(I)$]. Flack parameter = 0.00(4).

*Chrodrimanin A (**3**):* $[\alpha]^{26}_D$ +10 (*c* 0.1, MeOH−$CHCl_3$ = 1:1) [lit. $[\alpha]^{28}_D$ +8.21 (*c* 0.098, MeOH−$CHCl_3$ = 1:1)].[6]

*Chrodrimanin B (**4**):* $[\alpha]^{26}_D$ −39 (*c* 0.1, MeOH−$CHCl_3$ = 1:1) [lit. $[\alpha]^{28}_D$ −41.11 (*c* 0.111, MeOH−$CHCl_3$ = 1:1)].[6]

*Chrodrimanin E (**5**):* $[\alpha]^{26}_D$ −52 (*c* 0.1, MeOH−$CHCl_3$ = 1:1) [lit. $[\alpha]^{28}_D$ −51.6 (*c* 0.064, MeOH−$CHCl_3$ = 1:1)].[1]

*Chrodrimanin F (**6**):* $[\alpha]^{26}_D$ −64 (*c* 0.1, MeOH−$CHCl_3$ = 1:1) [lit. $[\alpha]^{28}_D$ −62.5 (*c* 0.032, MeOH−$CHCl_3$ = 1:1)].[1]

*Chrodrimanin F (**7**):* $[\alpha]^{26}_D$ −14 (*c* 0.1, MeOH−$CHCl_3$ = 1:1) [lit. $[\alpha]^{28}_D$ −14.2 (*c* 0.092, MeOH−$CHCl_3$ = 1:1)].[1]

X-ray Crystallographic Analysis of 1 and 2. Single-crystal X-ray diffraction data were collected on an Agilent Gemini Ultra diffractometer with Cu Kα radiation (λ = 1.541 84 Å). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined relatively isotropically with a riding model. A crystal suitable for X-ray diffraction was obtained by

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slow evaporation of a solution in MeOH−H_2O. Crystallographic data for **1** and **2** have been deposited in the Cambridge Crystallographic Data Centre with the deposition numbers 1029896 and 1062419. Copies of the data can be obtained, free of charge, from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data-request/cif.

■ ASSOCIATED CONTENT

Supporting Information

HRESIMS, NMR, and ECD spectra of compounds **1** and **2**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00103.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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