Coniochaetones E–I, new 4H-chromen-4-one derivatives from the Cordyceps-colonizing fungus Fimetariella sp.

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A B S T R A C T
Five new 4H-chromen-4-one derivatives coniochaetones E–I (1–5), along with the known compounds coniochaetones B (6) and A (7) have been isolated from solid cultures of the Cordyceps-colonizing fungus Fimetariella sp. Their structures were elucidated primarily by NMR spectroscopy and the absolute configurations of compounds 1–3 were assigned using the modified Mosher’s method. Compound 4 showed weak cytotoxic activity against HeLa cells with IC₅₀ values of 72.8 μM. The co-isolated known compound 6 showed modest inhibitory effects against Aspergillus fumigates, Fusarium oxysporum and Fusarium nivale.

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1. Introduction

“DongChongXiaCao” (DCXC; winter-worm, summer-grass) or Chinese caterpillar fungus, has been used as a tonic for the weak in Chinese tradition and was honored as one of the three greatest invigorants together with ginseng and pilose antlers [1]. Actually, DCXC is the complex of the fungus Cordyceps sinensis (Berk.) Sacc (later reclassified as Ophiocordyceps sinensis) [2] and the dead caterpillar larva of the moth Hepialus spp., which was found at high altitudes on the Qinghai–Tibetan Plateau. However, some other fungal species, namely, Cordyceps-colonizing fungi, were isolated from the stromata and sclerotia of O. sinensis. It’s still a puzzle whether these fungi make contribution to the claimed medical benefits. We initiated chemical investigations of these fungi and obtained numbers of new bioactive natural products [3–5]. The strain Fimetariella sp. (S207) was isolated from a sample of O. sinensis collected in Kangding, Sichuan Province, P.R. China. Our prior investigation of this strain has resulted in the isolation of fimetarone A with the new spiro[chroman-3,7’-isochromene]-4,6’(8’H)-dione skeleton, which showed modest cytotoxicity against T24 cells [5]. Since its HPLC fingerprint revealed the presence of many other components, the fungus was fermented in a larger scale on rice. Its organic extract afforded five new 4H-chromen-4-one derivatives coniochaetones E–I (1–5), along with two known metabolites coniochaetones B (6) and A (7). Details of the isolation, structure elucidation and cytotoxicity evaluation of these compounds are reported herein.

2. Experimental

2.1. General

Optical rotations were measured on an Autopol IV polarimeter, and UV data were obtained on an Unico UV-2800A spectrophotometer. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. 1H and 13C NMR data were acquired with Bruker Avance-400 and -500 spectrometers using solvent signals (CDCl₃: δH 7.26/δC 77.6; DMSO-d₆: 2.04/39.53).
The culture of *Fimetariella* sp. (*Lasiosphaeriaceae*) was isolated from the fruiting body of *O. sinensis* collected from Kangding, Sichuan Province, People’s Republic of China, in May, 2005. The isolate was identified by one of the authors (X.L.) based on morphology and sequence (Genbank Accession No. JQ823120) analysis of the ITS region of the rDNA and assigned the accession number 05-6-z19 in X.L.’s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants for 30 min. After cooling to room temperature, each flask was processed as described above for 1a.

**2.2. Fungal material**

2.2.1. Isolation and Purification

The fermented rice substrate was extracted with EtOAc (5 × 500 mL), and the organic solvent was evaporated to dryness under reduced pressure to afford a crude extract (30.0 g), which was fractionated by silica gel VLC using petroleum ether–EtOAc gradient elution. The fraction (630 mg) eluted with 50% EtOAc was separated by Sephadex LH-20 CC eluting with MeOH, and then the fractions were purified by RP HPLC (58% CH3CN in H2O for 20 min; 2 mL/min) to afford 1 (5.4 mg, tR 29.4 min; 20% CH3CN in H2O for 2 min, followed by 20–43% CH3CN over 40 min; 2 mL/min). The fraction (460 mg) eluted with 30% EtOAc was separated by Sephadex LH-20 CC eluting with MeOH, and further purification of the resulting subfractions by RP HPLC to afford 2 (0.5 mg, tR 26.9 min; 2 mL/min) and 3 (0.1 mg, tR 30.0 min; 2 mL/min).

**2.2.2. Fungal material**

The culture of *Fimetariella* sp. (*Lasiosphaeriaceae*) was isolated from the fruiting body of *O. sinensis* collected from Kangding, Sichuan Province, People’s Republic of China, in May, 2005. The isolate was identified by one of the authors (X.L.) based on morphology and sequence (Genbank Accession No. JQ823120) analysis of the ITS region of the rDNA and assigned the accession number 05-6-z19 in X.L.’s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants for 30 min. After cooling to room temperature, each flask was processed as described above for 1a.

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HRESIMS m/z 271.0583 [M + Na]^+ (calcd for C_{13}H_{12}O_5Na, 271.0577).

Preparation of (S)-(2a) and (R)-MTPA (2b) Esters. A sample of 2 (1.0 mg, 0.004 mmol) was dissolved in 200 µL anhydrous pyridine in a 5 mL vial. (R)-MTPA CI (0.8 µL, 0.004 mmol) was quickly added, the vial was sealed, and the mixture was kept at ambient temperature for 12 h. The mixture was evaporated to dryness and purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 µm; 9.4 x 250 mm; 50% MeOH in H2O for 2 min, followed by 50–100% MeOH over 20 min, then kept 100% MeOH for 10 min; 2 mL/min) to afford the monoester 2a (0.3 mg, tR 22.6 min): white powder; \(^1\)H NMR (DMSO-d_{6}, 500 MHz) \(\delta\) 12.19 (1H, s, 10-OH), 7.00 (1H, br s, H-7), 6.74 (1H, br s, H-9), 6.47 (1H, d, \(J = 6.1\) Hz, 3-OH), 6.18 (1H, d, \(J = 7.0\) Hz, H-1), 5.16 (1H, m, H-3), 2.40 (3H, s, H3-14), 2.36 (1H, m, H-2a), 2.30 (1H, dd, \(J = 14.4, 7.9\) Hz, H-2b).

In a similar fashion, a sample of 2 (1.0 mg, 0.004 mmol), (S)-MTPA CI (0.8 µL, 0.004 mmol) and anhydrous pyridine (200 µL) were allowed to react in a 5 mL vial at ambient temperature for 12 h, and the reaction mixture was processed as described above for 2a to afford the monoester 2b (0.3 mg, tR 21.8 min): white powder; \(^1\)H NMR (DMSO-d_{6}, 500 MHz) \(\delta\) 12.19 (1H, s, 10-OH), 7.00 (1H, br s, H-7), 6.73 (1H, br s, H-9), 6.53 (1H, d, \(J = 6.7\) Hz, 3-OH), 6.20 (1H, d, \(J = 7.2\) Hz, H-1), 5.32 (1H, m, H-3), 2.39 (3H, s, H3-14), 2.50 (1H, overlapped with the DMSO peak, H-2a), 2.36 (1H, dd, \(J = 12.5, 6.3\) Hz, H-2b).

Coniochaetone G (3): white powder; [\(\alpha\)]\(^{19}\)D = +94.0 (c 0.1, MeOH); UV (MeOH) \(\lambda_{\text{max}}\) (log e) 241 (3.08), 329 (2.34) nm; IR (neat) \(v_{\text{max}}\) 3400 (br), 1658, 1321, 1212, 1040 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data see Table 1; HMBC data (DMSO-d_{6}, 400 MHz) \(^1\)H-1 \(\rightarrow\) C-9, 10, 11; NOESY correlations (DMSO-d_{6}, 400 MHz) H-2a \(\rightarrow\) H-1, H-3; H-2b \(\rightarrow\) H-7, H-9; HRESIMS m/z 271.0581 [M + Na]^+ (calcd for C_{13}H_{12}O_5Na, 271.0577).

Preparation of (S)-(3a) and (R)-MTPA (3b) Esters. A sample of 3 (1.0 mg, 0.004 mmol) was dissolved in 200 µL anhydrous pyridine in a 5 mL vial. (R)-MTPA CI (0.8 µL, 0.004 mmol) was quickly added, the vial was sealed, and the mixture was kept at ambient temperature for 12 h. The mixture was evaporated to dryness and purified by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 µm; 9.4 x 250 mm; 50% MeOH in H2O for 2 min, followed by 50–100% MeOH over 20 min, then kept 100% MeOH for 10 min; 2 mL/min) to afford the monoester 3a (0.3 mg, tR 21.7 min): white powder; \(^1\)H NMR (DMSO-d_{6}, 500 MHz) \(\delta\) 12.23 (1H, s, 10-OH), 7.00 (1H, br s, H-7), 6.74 (1H, br s, H-9), 6.26 (1H, d, \(J = 6.3\) Hz, 3-OH), 6.24 (1H, dd, \(J = 7.6, 3.7\) Hz, H-1), 4.94 (1H, m, H-3), 3.11 (1H, ddd, \(J = 14.5, 7.6, 7.6\) Hz, H-2a), 2.40 (3H, s, H3-14), 1.54 (1H, ddd, \(J = 14.4, 3.7, 3.7\) Hz, H-2b).

In a similar fashion, a sample of 3 (1.0 mg, 0.004 mmol), (S)-MTPA CI (0.8 µL, 0.004 mmol) and anhydrous pyridine (200 µL) were allowed to react in a 5 mL vial at ambient temperature for 12 h, and the reaction mixture was processed as described above for 3a to afford the monoester 3b (0.3 mg, tR 21.2 min): white powder; \(^1\)H NMR (DMSO-d_{6}, 500 MHz) \(\delta\) 12.21 (1H, s, 10-OH), 7.00 (1H, br s, H-7), 6.72 (1H, br s, H-9), 6.32 (1H, d, \(J = 6.7\) Hz, 3-OH), 6.31 (1H, d, \(J = 6.8\) Hz, H-7), 4.97 (1H, m, H-3), 3.30 (1H, overlapped with the water peak, H-2a), 2.39 (3H, s, H3-14), 1.79 (1H, ddd, \(J = 14.7, 3.3, 3.3\) Hz, H-2b).

Coniochaetone H (4): white powder; [\(\alpha\)]\(^{19}\)D = +56.4 (c 0.1, MeOH); UV (MeOH) \(\lambda_{\text{max}}\) (log e) 243 (3.37), 329 (2.60) nm; IR (neat) \(v_{\text{max}}\) 3427, 3308, 1647, 1447, 1278, 1086 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR and HMBC data see Table 2; HRESIMS m/z 289.0240 [M + Na]^+ (calcd for C_{13}H_{12}O_5ClNa, 289.0238).

Coniochaetone I (5): white powder; UV (MeOH) \(\lambda_{\text{max}}\) (log e) 243 (4.20), 333 (3.41) nm; IR (neat) \(v_{\text{max}}\) 3347 (br), 2952, 2880, 1735, 1653, 1512, 1290, 1150 cm\(^{-1}\).
Table 2
NMR data of coniochaetones H (4) and I (5) in CDCl3.

<table>
<thead>
<tr>
<th></th>
<th>Coniochaetone H (4)</th>
<th>Coniochaetone I (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta_{1H}$ (Hz)</td>
<td>$\delta_{13C}$</td>
</tr>
<tr>
<td></td>
<td>$\delta_{1H}$, mult.</td>
<td>HMBC</td>
</tr>
<tr>
<td>1</td>
<td>5.46, dddd (7.6, 2.2, 1.1)</td>
<td>71.0, CH</td>
</tr>
<tr>
<td>2a</td>
<td>2.53, m</td>
<td>29.4, CH$_2$</td>
</tr>
<tr>
<td>2b</td>
<td>2.06, m</td>
<td>3.4</td>
</tr>
<tr>
<td>3a</td>
<td>3.14, dddd (18.1, 9.3, 5.0, 1.1)</td>
<td>30.0, CH$_2$</td>
</tr>
<tr>
<td>3b</td>
<td>2.85, dddd (18.1, 9.3, 5.0)</td>
<td>1, 2, 4, 13</td>
</tr>
<tr>
<td>4</td>
<td>172.6, qC</td>
<td>6, 9, 11, 12, 14</td>
</tr>
<tr>
<td>6</td>
<td>155.3, qC</td>
<td>144.5, qC</td>
</tr>
<tr>
<td>7</td>
<td>108.8, CH</td>
<td>116.4, qC</td>
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<tr>
<td>8</td>
<td>144.5, qC</td>
<td>156.2, qC</td>
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<tr>
<td>9</td>
<td>109.6, qC</td>
<td>109.6, qC</td>
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<td>10</td>
<td>180.8, qC</td>
<td>121.2, qC</td>
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<tr>
<td>11</td>
<td>7.3, CH$_3$</td>
<td>7, 8, 9</td>
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<td>12</td>
<td>21.3, CH$_3$</td>
<td>2.17, s</td>
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<tr>
<td>13</td>
<td>2.48, s</td>
<td>9, 10, 11</td>
</tr>
<tr>
<td>14</td>
<td>10-OH</td>
<td>13.02, s</td>
</tr>
</tbody>
</table>

*a* Recorded at 500 MHz.
* Recorded at 125 MHz.
* Recorded at 400 MHz.
* Recorded at 100 MHz.

2903, 1735, 1650, 1455, 1162, 1035 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 2; HMBC data (CDCl$_3$, 400 MHz) H$_2$-2 → C-1, 3, 4; H$_2$-3 → C-1, 2, 4, 13; H-7 → C-6, 8, 9, 11, 12, 14; H-9 → C-7, 10, 11, 12, 14; H$_2$-14 → C-7, 8, 9, 11; H$_2$-2′ → C-1′; 10-OH → C-9, 10, 11; HRESIMS m/z 289.0713 [M + H]$^+$ (calcd for C$_{13}$H$_{15}$O$_6$, 289.0707).

2.4. Antibacterial and antifungal bioassays

Antibacterial and antifungal bioassays were conducted in triplicate by following the National Center for Clinical Laboratory Standards (NCCLS) recommendations [6,7]. The bacterial strains *Staphylococcus aureus* col (CGMCC 1.1692), *Escherichia coli* (CGMCC 1.2340), *Enterococcus faecium* (CGMCC 1.2025), *Enterococcus faecalis* 850E (CGMCC 1.2135) and *Bacillus subtilis* (ATCC 6633) were grown on Mueller-Hinton agar, the yeasts, *Candida albicans* (CGMCC 2.2086) and *Saccharomycyes cerevisiae* (ATCC 18824), were grown on Sabouraud dextrose agar, the fungi, *Aspergillus fumigatus* (CGMCC 3.5835) and *A. flavus* (CGMCC 3.0951), as well as the plant pathogenic fungi, *Fusarium oxysporum* (CGMCC 3.2830), *F. moniliforme* (CGMCC 3.2835), *F. nivale* (CGMCC 3.4600), *Gibberella zeae* (CGMCC 3.2873) and *Alternaria longipes* (CGMCC 3.2875) were grown on potato dextrose agar. Targeted microbes (3–4 colonies) were prepared from broth culture (bacteria: 37 °C for 24 h; fungus: 28 °C for 48 h), and the final spore suspensions of bacteria (in MHB medium), of yeasts (in SDB medium), and fungi (in PDB medium) were 10$^6$, 10$^5$ cells/mL and 10$^6$ mycelial fragments/mL, respectively. Test samples (10 mg/mL as stock solution in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspension of the test organisms was added to each well, achieving a final volume of 200 μL (ampicillin was used as the positive control for the Gram-positive bacteria, gentamycin for the Gram-negative bacteria, amphotericin B for the yeasts, the fungi *A. fumigates* and *A. flavus*, carbendazim for the plant pathogenic fungi, respectively). After incubation, the absorbance at 595 nm was measured with a microplate reader (Tecan). The MIC was defined as the lowest test concentration that completely inhibited the growth of the test organisms.

2.5. MTT assay [8]

The assay concentrations of the test compounds and the positive control cisplatin (100 mM as stock solution was run in triplicate. In a 96-well plate, each well was plated with (2 − 5) × 10$^3$ cells (depending on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 100 μL of medium containing 0.1% DMSO or appropriate of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). The plate was incubated for 48 h at 37 °C in a humidified, 5% CO$_2$ atmosphere. Proliferation was assessed by adding 20 μL of MTS (Promega) to each well in the dark, followed by 90 min incubation at 37 °C. The assay plate was read at 490 nm using a microplate reader. The inhibition was calculated and plotted versus test concentrations to afford the IC$_{50}$.

3. Results and discussion

The elemental composition of coniochaetone E (1) was established as C$_{15}$H$_{14}$O$_7$ by HRESIMS (m/z 329.0639 [M + Na]$^+$; Δ −0.7 mmu). Its $^1$H and $^{13}$C NMR spectra showed resonances for one intramolecularly hydrogen-bonded hydroxyl group ($\delta_{1H}$ 11.94), two exchangeable protons ($\delta_{1H}$ 4.15 and 2.62, respectively), one O-methyl, one aromatic methyl, one isolated methane, one oxygenated methane, one oxygenated
sp³ carbon, eight aromatic/olefinic carbons (three of which are oxygenated, two protonated), one carboxylic carbon (δC 174.1), and one α,β-unsaturated ketone carbon (δC 180.5). The two meta-coupled aromatic protons (δH 5.65 and 6.80, respectively) implied the presence of an 1,2,3,5-tetrasubstituted benzene ring. The methyl group (δH 2.41/22.5) was located at C-8 between the two protonated aromatic carbons based on HMBC correlations from H2-14 to C-7, C-8 and C-9. The phenolic hydroxyl group (δH 11.94) was attached to C-10 accounting for the low-field chemical shift of C-10 (δC 161.0) and the HMBC correlations from 10-OH to C-9, C-10 and C-11. The ketone group (δC 180.5) should be located ortho to the phenolic hydroxyl group considering the ¹H chemical shift of 10-OH and the four-bond W-type correlation from H-9 to C-12 [9]. The HMBC correlations from H2-2 to C-1, C-3, C-4 (δC 169.6) and C-13 (δC 120.4), and H-3 to C-1, C-4 and C-13, and 3-OH (δH 2.62) to C-2 and C-3 established the 3-hydroxycyclopentene moiety. The remaining hydroxyl group (δH 4.15) and the methyl formate unit (δC 381.538/174.1) were both attached to C-1 supported by the HMBC correlations from 1-OH to C-1, C-2, C-13 and C-1’ and from H2-2’ to C-1’. Considering the chemical shifts of C-4 (δC 169.6), C-6 (δC 157.6) and C-13 (δC 120.4) and the unsaturation requirement of 1, C-4 and C-6 should be attached to the remaining oxygen atom with C-13 attached to C-12 to form the pyran-4-one moiety. On the basis of these data, the planar structure of 1 was established as shown (Fig. 1).

The relative configuration of 1 was assigned by analysis of its ROESY data. ROESY correlations between the exchangeable proton 1-OH with H-2b and 3-OH, H-2b with 3-OH, H2-2’ with H-2a indicated that the two hydroxyl groups are on the same side of the cyclopentene ring. The absolute configuration of the C-3 secondary alcohol in 1 was determined by application of the modified Mosher’s method [10]. Compound 1 was treated with R-(−)- and S-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (MTPA-Cl), respectively, and the corresponding S- and R-Mosher monoesters 1a and 1b was obtained. The reaction products were selective derivatives of 3-OH, which may account for the less steric hindrance for 3-OH. Interpretation of the ¹H NMR chemical shift differences (Δδ = δCr − δCs) between 1a and 1b, established the absolute configuration of C-3 as S by applying Mosher ester analysis (Fig. 2). Considering the relative configuration determined by the ROESY date, compound 1 was assigned the 1R, 3S absolute configuration.

Coniochaetone F (2) was given the molecular formula C13H12O5 on the basis of its HRESIMS data (m/z 271.0583 [M + Na]+; Δ − 0.6 mmu), which is 58 mass units less than 1. Its ¹H and ¹³C NMR data (Table 1) revealed structural fragments similar to those in 1, except that the resonances for the methyl formate unit (δH 3.81/53.8, 174.1) was replaced by the resonances for the oxygenated methine proton (δH 5.10). These observations were confirmed by the HMBC correlations from 1-OH (δH 5.20) to C-1, C-2 and C-13 and H-3 to C-3, C-4 and C-13. The ¹H-¹H COSY NMR data showed the isolated spin systems of C-1-C-3 (including 1-OH and 3-OH), which further supported this assumption. The relative configuration of 2 was deduced on the basis of its NOESY data. NOESY correlations of H-1 with H-2b and H-3 with H-2a placed H-1 on the face opposite of H-3. The absolute configuration of the C-1 secondary alcohol in 2 was determined using the modified Mosher’s method. Treatment of 2 with (R)- and (S)-MTPA Cl afforded the major products S-(2a) and R-MTPA (2b) monoesters, respectively. The differences in chemical shift values (Δδ = δCr − δCs) for the diastereomeric esters 2a and 2b was calculated to assign the 1S, 3R absolute configuration (Fig. 2). Therefore, the 1S, 3R absolute configuration was deduced for 2.

Table 3

<table>
<thead>
<tr>
<th>Comp</th>
<th>MIC (μg/mL)</th>
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<tbody>
<tr>
<td></td>
<td>A. <em>fumigatus</em></td>
</tr>
<tr>
<td>6</td>
<td>5.00</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>10.00</td>
</tr>
<tr>
<td>Carbendazim</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Structure for compounds 1–7.
Coniochaetone G (3) was assigned the same molecular formula C13H12O5 as 2 by HRESIMS (m/z 271.0581 [M + H]+; δ –0.4 mmu). Analysis of its 1H, 13C, HMBC and 1H-1H COSY NMR spectra indicated the same planar structure as 2. The varieties of NMR data in 3 (Table 1) were caused by the different stereochemistry of C-1 or C-3. NOESY correlations of H-2α with H-1 and H-3 displayed that H-1 and H-3 are on the same side of the cyclopentene ring. The absolute configuration of the C-1 secondary alcohol in 3 was also determined using the modified Mosher’s method. The differences in chemical shift values (Δδ = δS – δR) for its S-((3a) and R-MTPA (3b) monoesters indicated the 1S absolute configuration of 3 (Fig. 2). Therefore, the 1S, 3R absolute configuration was proposed for 3.

Coniochaetone H (4) gave a pseudo molecular ion [M + Na]+ peak at m/z 289.0240 (δ –0.2 mmu) by HRESIMS, consistent with the molecular formula C13H11O4Cl. Its 1H and 13C NMR spectra showed resonances for one intramolecularly hydrogen-bonded hydroxyl group (δH 13.02), one methyl, two methylenes, one oxygenated methine, eight aromatic/olefinic carbons (three of which are oxygenated, one protonated) and one αβ-unsaturated ketone carbons (δC 180.8). These data accounted for all the NMR resonances of 4 except for one exchangeable proton. The 1H and 13C NMR data of 4 (Table 2) are similar to those of the known compound coniochaetone B (6) [11], except that one of the two aromatic protons in 6 was replaced by a chlorine atom in 4. The chlorine atom was attached to C-9 (δC 116.4 in 4; δH/δC 6.62/112.6 in 6, both in CDCl3) confirmed by the HMBC correlations from H3-14 to C-7, C-8 and C-9, H-7 to C-6, C-9, C-11, C-12 and C-14 and the phenolic hydroxyl proton 10-OH to C-9, C-10 and C-11. The absolute configuration of C-1 in 4 was proposed as R according to the positive sign of the optical rotation ([α]D + 54.6, c 0.1, MeOH), when comparing to that of (R)-coniochaetone B ([α]D + 84.0, c 0.1, MeOH) [13] and (S)-coniochaetones C ([α]D − 49.0, c 0.1, MeOH) and D ([α]D − 46.8, c 0.1, MeOH) [15]. On the basis of the above evidence, the structure of 4 was assigned as described.

Coniochaetone I (5) was assigned the molecular formula C15H12O6 by HRESIMS (m/z 289.0713 [M + H]+; δ –0.6 mmu). Analysis of its NMR data of 5 (Table 2) revealed the presence of structural features similar to those of the known compound coniochaetone A (7) [11], except that the C-8 methyl group (δH/δC 2.41/22.4) was replaced by the acetylated hydroxymethyl unit (δH/δC 5.14/64.7; 2.17/20.8; 170.4), which was supported by HMBC correlations from H2-14 and H3-2′ to C-1′ and from H2-14 to C-7, C-8 and C-9. Collectively, the structure of 5 was determined as shown.

![Scheme 1. Hypothetical biosynthetic pathways for coniochaetones E-I (1-7).](image-url)
Compounds 6 and 7 were identified as coniochaetones B and A, the secondary metabolites primarily isolated from the coprophilous fungus \textit{Coniochaeta saccardoi}, by comparison of its NMR and MS data with literature values [11].

Compounds 1–7 were evaluated for antibacterial activity against \textit{Staphylococcus aureus} col (CGMCC 1.2465), \textit{Streptococcus pneumoniae} (CGMCC 1.1692), \textit{Escherichia coli} (CGMCC 1.2340), \textit{Enterococcus faecium} (CGMCC 1.2025), \textit{E. faecalis} 850E (CGMCC 1.2135) and \textit{Bacillus subtilis} (ATCC 6633), for antifungal activity against a panel of fungi \textit{Candida albicans} (CGMCC 2.2086), \textit{Saccharomyces cerevisiae} (ATCC 18824), \textit{Aspergillus fumigatus} (CGMCC 3.5835), \textit{A. flavus} (CGMCC 3.0951), \textit{Fusarium oxysporum} (CGMCC 3.2830), \textit{F. moniliforme} (CGMCC 3.2835), \textit{F. nivale} (CGMCC 3.4600), \textit{Gibberella zeae} (CGMCC 3.2873) and \textit{Alternaria longipes} (CGMCC 3.2875) and for cytotoxicity against the human cancer cell lines, HeLa (cervical epithelial cells), A549 (lung carcinoma epithelial cells), HCT116 (colon cancer cells), SW480 (colon cancer cells) and T24 (human bladder carcinoma cells). The known compound coniochaetone B (6) showed inhibitory effects against most of the test fungi strains (Table 3). Coniochaetone B (6), as well as coniochaetone A (7), showed no inhibitory effect against \textit{C. albicans} and \textit{S. cerevisiae} at the concentration of 20 μg/ml although they were claimed active in a standard disk assay against \textit{C. albicans} [11]. Compound 4 exhibited weak cytotoxic activity against HeLa cells with IC_{50} value of 72.8 μM (the positive control cisplatin showed an IC_{50} value of 11.9 μM).

Coniochaetones E–I (1–5) are five new members of naturally rare tricyclic cyclopentabenzopyran-9-ones. Compound 1 is structurally related to remisporine A, which is not stable under normal conditions and dimerized readily to remisporine B [12]. The hydration of the double bond C-2/C-3 in 1 makes the molecule more stable. The other analogues of 1 include coniothyrione [13] and preussochromones D–F, which possess different substituents on the aryl and cyclopentane rings [14]. Compounds 2–4 differ from the reported compounds coniochaetones B [11], C and D by having different substituents either in the aryl rings or in the cyclopentane rings [15]. Compound 4 is the only one with chlorine substitution on the aryl ring in the reported natural tricyclic cyclopentabenzopyran-9-ones. Compound 5 and coniochaetone A possess the same cyclopentabenzopyran-1, 9-dione core structure but different substituents at C-8 [11]. The proposed biosynthetic pathway of coniochaetones E–I was illustrated in Scheme 1. One of the aryl rings of the xanthone 4,8-dihydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (8), as the presumed precursor, could be transformed into a cyclopentane ring by a series of oxidation, isomerization, hydrolysis, ring closure and decarboxylation. The similar transformation could be observed in the biosynthesis from O-methylsterigmatocystin to aflatoxin B1 [16].

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Reference