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A Spiro[chroman-3,7′-isochromene]-4,6′(8′*H*)-dione from the *Cordyceps*-Colonizing Fungus *Fimetariella* sp.

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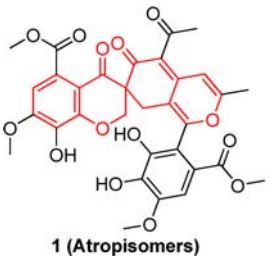
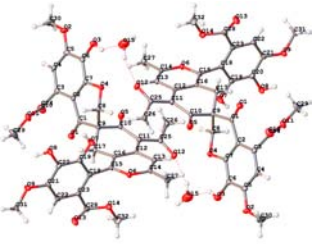
ABSTRACT



1 (Atropisomers)



Fimetarone A (1), a metabolite with the new spiro[chroman-3,7′-isochromene]-4,6′(8′*H*)-dione skeleton, was isolated from cultures of the *Cordyceps*-colonizing fungus *Fimetariella* sp. Compound 1 was a 1:1 atropdiastereomeric mixture in NMR data, and a*S*,9*S* and a*R*,9*R* enantiomers were found and confirmed by X-ray crystallography. Compound 1 could be derived from the hypothetical precursors 3,4,5-trihydroxy-2-(2-methylene-3,5-dioxohexanoyl)benzoic acid (5) and lapidosin (6).

Cordyceps sinensis (Berk.) Sacc. (later reclassified as *Ophiocordyceps sinensis*),[1] known as Chinese caterpillar fungus or "Dong Chong Xia Cao" (caterpillar-in-winter, herb-in-summer), is actually the combination of the fungus and the dead caterpillar larva of the moth *Hepialus* spp. which was found primarily at high altitudes on the Himalayan Plateau. It has been used for centuries in traditional Chinese medicine (TCM), showing extensive nutritional and medicinal effects.[2] However, due to the claimed health benefits and growing popularity, the natural *C. sinensis* has been overharvested to the status of an endangered species. The scarcity of the natural material has limited the in-depth chemical investigations of the fungus for bioactive natural products.

The search for bioactive natural products from fungi of unique ecological niches has proven effective.[3] The fungal species that colonize the fruiting body and larvae of *C. sinensis*, namely, *Cordyceps*-colonizing fungi, could be a valuable and alternative source of bioactive secondary metabolites with ecological implications. On the basis of this consideration, we have initiated chemical studies of the *Cordyceps*-colonizing fungi isolated from *C. sinensis* samples found in alpine regions of Tibet and Sichuan.[4] In the current work, the strain *Fimetariella* sp. (S207) was isolated from a sample of *C. sinensis* collected in Kangding,

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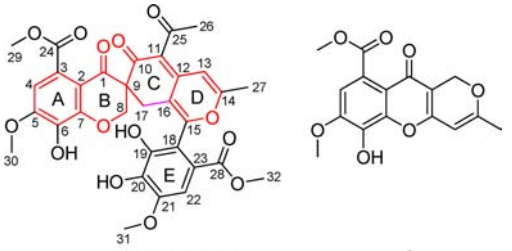
Sichuan Province, P. R. China, and grown in a solid substrate fermentation culture. Its EtOAc extract was cytotoxic against human tumor cell lines, HeLa (cervical epithelial cells), A549 (lung carcinoma epithelial cells), and T24 (bladder carcinoma cells). Fractionation of the extract led to the isolation of fimetarone A (**1**), a unique natural product with the new spiro[chroman-3,7′-isochromene]-4,6′(8′*H*)-dione core, together with the known compound chaetocyclinone B (**2**).[5] Details of the structure elucidation, cytotoxicity evaluation, and plausible biogenesis of **1** are reported herein.



1 (1a and 1b) **2**

Fimetarone A (**1**) was obtained as a mixture of two atropisomers (**1a** and **1b**) in a ratio of approximately 1:1, as determined by integration of some well-resolved ^{1}H NMR resonances for each isomer. Although the two isomers were well-resolved on an HPLC column, analysis of each collected peak revealed the presence of both isomers, suggesting the occurrence of a spontaneous equilibration. Therefore, the structure was elucidated on the equilibium mixture. Compound **1** gave a protonated molecule $[M + H]^+$ peak by HRESIMS, consistent with the molecular formula $C_{32}H_{28}O_{14}$ (19 degrees of unsaturation). The ^{1}H and ^{13}C NMR spectra of **1a** showed resonances for six methyl groups with four *O*-methyls, two methylenes (one of which is oxygenated), 18 aromatic/olefinic carbons with three protonated, one sp^3 quaternary carbon, two carboxylic carbons (δ_C 165.2 and 169.3, respectively), and three α,β-unsaturated ketone carbons (δ_C 190.1, 192.5, and 199.0). These accounted for all of the NMR resonances of **1a** except for three exchangeable protons (OH-6, -19, and -20) and required **1a** to be a pentacyclic compound. Interpretation of the NMR data of **1a** (Table 1) revealed two pentasubstituted aryl rings (A and E). The substructure of ring A with C-4 protonated was confirmed by HMBC cross peaks from H-4 to the sp^2 carbons C-1 (a four-bond *W*-type correlation),[5] C-2, C-3, C-5, C-6, and C-24, H_3-29 to C-24, and from H_3-30 to C-5, whereas the presence of ring E was supported by HMBC correlations from H-22 to the sp^2 carbons C-18, C-19, C-20, C-21, C-23, and C-28, H_3-31 to C-21, and from H_3-32 to C-28 (Figure 1). In addition, NOESY correlations of H-4 with H_3-29 and H_3-30 and of H-22 with H_3-31 and H_3-32 indicated that the C-24 methyl formate and C-30 methoxy were adjacent to H-4, and the C-28 methyl formate and C-31 methoxy were adjacent to H-22. The C-24 methyl formate attached to C-3 was partially supported by the ^{13}C NMR chemical shift (δ_C 113.7) for C-2.[6]

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Table 1. NMR Data of **1a** in DMSO-d_6

pos.	δ_H[a] (*J* in Hz)	δ_C[b]	HMBC (H → C#)
1		190.1	
2		113.7[c]	
3		124.8	
4	6.72, s	105.2	1, 2, 3, 5, 6, 24
5		151.9	
6		135.0	
7		150.2	
8a	4.32, d (11.8)	72.9	1, 7, 9, 10, 17
8b	4.17, d (11.8)		1, 7, 9, 10, 17
9		53.0	
10		192.5	
11		113.3[c]	
12		149.0	
13	7.50, br s	105.9	11, 14, 16, 27
14		163.1	
15		157.2	
16		113.4[c]	
17a	3.16, d (16.2)	25.9	1, 8, 9, 10, 12, 15, 16
17b	2.17, d (16.2)		1, 8, 9, 10, 12, 15, 16
18		113.3[c]	
19		144.2	
20		139.0	
21		148.5	
22	7.20, s	106.1	18, 19, 20, 21, 23, 28
23		119.1	
24		169.3	
25		199.0	
26	2.25, s	32.1	11, 25
27	2.30, br s	19.7	13, 14
28		165.2	
29	3.70, s	52.1	24
30	3.83, s	56.3	5
31	3.86, s	56.1	21
32	3.69, s	52.1	28

[a] Recorded at 600 MHz. [b] Recorded at 150 MHz. [c] These assignments are interchangeable.

Furthermore, the C-28 methyl formate was located at C-23 by a NOESY correlation of H_3-27 with H_3-32. The subunit of ring B fused to ring A via C-2−C-7 was established by HMBC cross peaks from H_2-8 to C-1 (δ_C 190.1), C-7, and C-9 and from H-4 to C-1. In turn, correlations from H_2-17 to C-9, C-10 (δ_C 192.5), C-12, and C-16, H_3-26 to C-11 and C-25 and from H-13 to C-11 and C-16 assigned a cyclohexenone moiety (ring C) with the acetyl group attach to C-11. The spiro-junction for rings B and C was unambiguously assigned by HMBC cross peaks of H_2-8 and H_2-17 with C-1, C-9, and C-10. Further correlations from H_2-17 to C-15 and

(6) For chroman(en)-4-one, the C-5 was substituted by formate, the C-4a chemical shift (δ_C > 113 ppm) was downfield corresponding to C-5 substituted by hydroxy group (δ_C < 109 ppm). See: (a) Capon, R. J.; Stewart, M.; Ratnayake, R.; Lacey, E.; Gill, J. H. *J. Nat. Prod.* **2007**, *70*, 1746–1752. (b) Conrad, J.; Foerster-Fromme, B.; Constantin, M. A.; Ondrus, V.; Mika, S.; Mert-Balci, F.; Klaiber, I.; Pfannstiel, J.; Moeller, W.; Rosner, H.; Foerster-Fromme, K.; Beifuss, U. *J. Nat. Prod.* **2009**, *72*, 835–840. (c) Mutanyatta, J.; Matapa, B. G.; Shushu, D. D.; Abegaz, B. M. *Phytochemistry* **2003**, *62*, 797–804. (d) Kong, F.; Carter, G. T. *Tetrahedron Lett.* **2003**, *44*, 3119–3122.

from H-13 to C-11, C-14, and C-16, plus the chemical shifts for C-14 (δ_C 163.1) and C-15 (δ_C 157.2), connected C-14 and C-15 to the same oxygen atom to form the spiro[chroman-3,7′-isochromene]-4,6′(8′*H*)-dione core for **1a**. The C-27 methyl group was located at C-14 by HMBC correlations from H_3-27 to C-13 and C-14. The C-15−C-18 linkage for rings D and E was only supported by a NOESY correlation of H_3-27 with H_3-32. The three exchangeable protons in **1a** were attached to C-6, C-19, and C-20, respectively, by default, which were partially supported by the ^{13}C NMR data for C-6 (δ_C 135.0), C-19 (δ_C 144.2), and C-20 (δ_C 139.0). Collectively, the planar structure of **1** was tentatively assigned as shown.

The proposed structure for **1** was confirmed by single-crystal X-ray diffraction analysis (Figure 2). Compound **1** was found to crystallize as a mixture of the enantiomers (a*S*,9*S* and a*R*,9*R*), consistent with its zero optical rotation value and CD spectrum (Figure S5). Subsequent HPLC separation of the enantiomers using chiral stationary phases was unsuccessful (Figures S6−S13). To our knowledge, fungal natural products isopestacin, pestacin, and pestalachloride A[7−9] have been reported as racemic mixtures of the *S* and *R* enantiomers.

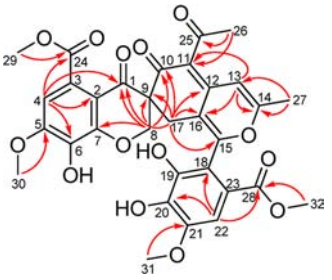

Figure 1. Selected key HMBC correlations for **1**.

The observation of two isomers in the NMR spectra of **1** was likely due to the hindered rotation around the C-15−C-18 axis.[10] To assess the energy barrier for the atropisomers of **1**, variable-temperature ^1H NMR spectra were recorded at various temperatures (Figures S2 and S3). All of the ^1H NMR spectra acquired at different temperatures showed signals for the two isomers in a ratio of approximately 1:1, indicating that the rotation of ring E around the C-15−C-18 axis is restricted for the a*S* and a*R* isomers due to the bulky methyl formate group at C-23. Therefore, **1** was assigned as an atropdiastereomeric mixture of the 9*S* and 9*R* enantiomers.

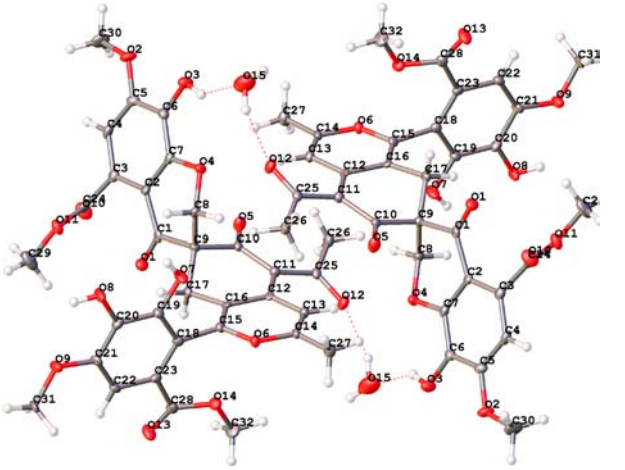

Figure 2. Thermal ellipsoid representation of **1**.

Compound **2** was identified as chaetocyclinone B, a secondary metabolite isolated from the endosymbiotic fungus *Chaetomium* sp., by comparison of its NMR and MS data with literature values.[5]

Compounds **1** and **2** were tested for cytotoxicity against human tumor cell lines, HeLa, A549, and T24. Compound **1** showed modest cytotoxicity against T24 cells, with an IC_{50} value of 40.2 μM. While compound **2** was cytotoxic to HeLa, A549, and T24 cells, showing IC_{50} values of 33.1, 20.1, and 5.38 μM, respectively (the positive control cisplatin showed an IC_{50} value of 4.06, 8.45, and 3.72 μM against the three cell lines).

Although several synthetic compounds with partial structure similarity to fimetarone A (**1**) have been previously reported, incoporating either a spiro[chroman-3,1′-cyclohexene]-dione skeleton[11] or a spiro[chroman-3,5′-[1,3]dioxan]-4-one core,[12] compound **1** possesses a novel structural feature in which a previously undescribed spiro[chroman-3,7′-isochromene]-4,6′(8′*H*)-dione moiety linked to a gallic acid unit via the C-15−C-18 bond.

From a biosynthetic aspect, compounds **1** and **2** could be generated from a hypothetical intermediate **4** derived

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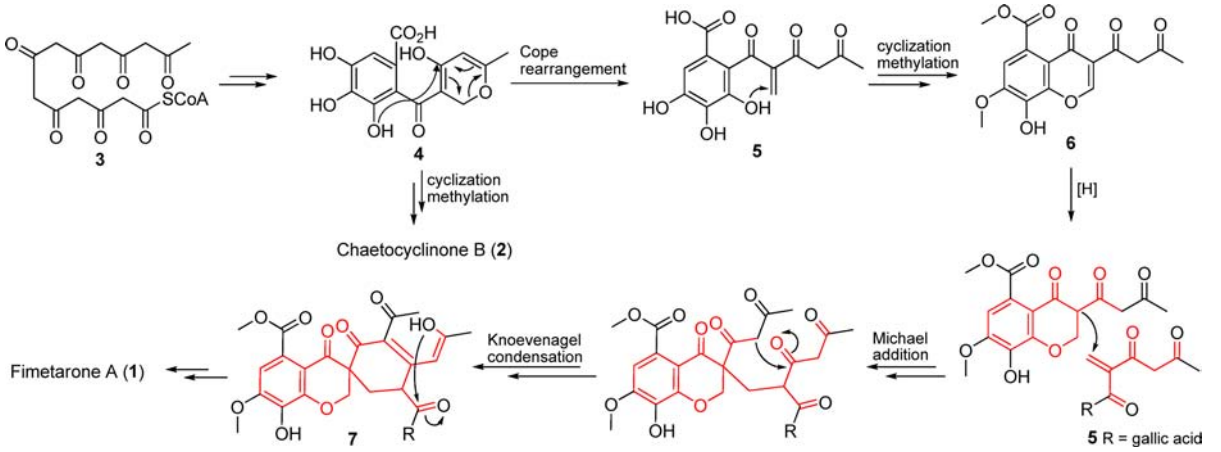
Scheme 1. Hypothetical Biosynthetic Pathways for **1** and **2**



from cyclization of a heptaketide **3**.[13a] Although 3,4,5-trihydroxy-2-(2-methylene-3,5-dioxohexanoyl)benzoic acid (**5**), a possible key intermediate initially proposed in the biosynthetic study of the fungal natural product polivione,[13] and the known compound lapidosin (**6**),[14] were not coisolated in the current work, they could be the hypothetical biosynthetic precursors leading to the formation of **1** via a series of reactions including Michael addition and Knoevenagel condensation,[15,16] as illustrated in the proposed plausible biosynthetic pathways shown in Scheme 1.

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Supporting Information Available. Experimental procedures, characterization data, ^{1}H, ^{13}C NMR, and CD spectra of **1**, and X-ray data of **1** (CIF file). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.