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Sporormiellin A, the first tetrahydrofuran-fused furochromone with an unprecedented tetracyclic skeleton from *Sporormiella minima*

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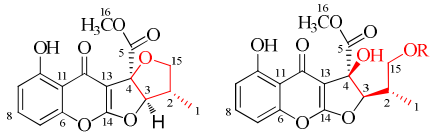
Sporormiellin A (1), the first tetrahydrofuran-fused furochromone with an unprecedented tetracyclic skeleton, has been obtained from the fungal strain *Sporormiella minima*. The structure with absolute configuration was elucidated by NMR data, X-ray crystallography, and quantum chemical ECD calculation.

2*H*-furo[2,3-b]chromen-4(3*H*)-ones are a subclass of furochromenones with diverse biological activities, such as inhibitory activity on nitric oxide-release,[1] anti-α-glucosidase,[2] anthelminthic,[3] antiplatelet aggregation,[4] anti-HIV replication,[4] cytotoxic,[5] and neuroprotective[6] activities, and so on. Until now, around 150 2*H*-furo[2,3-b]chromen-4(3*H*)-ones have been reported. Natural 2*H*-furo[2,3-b]chromen-4(3*H*)-ones possess a tricyclic skeleton (6/6/5, such as pallidones I and J,[7] fukanefurochromones,[1] coniofurol A[8]) or a benzene-fused tetracyclic skeleton (6/6/5/6, such as aervins A–C,[9] ayamenins,[10] euchretins[4]).

During our ongoing research on bioactive secondary metabolites from fungi,[11] a chemical investigation of metabolites from *Sporormiella minima* (No. 66-3-4-2) isolated from the lichen *Nephromopsis pallescens* (Schaer.) Y. S. Park was carried out, which led to the isolation of three novel linear pyran-furan fusion furochromenones, sporormiellins A (**1**)–C (**3**), and three biogenetically related compounds, sporormiellones A (**4**), B (**5**), and microsphaeropsone A (**6**) (Scheme 1). Interestingly, sporormiellin A (**1**) features an unprecedented tetracyclic skeleton core (6/6/5/5) and is the first case of tetrahydrofuran-fused furochromones. Details of the structure elucidation for **1**–**3** (Fig. 1) are reported herein. In addition, a plausible biogenetic pathway of **1**–**3** is proposed.

The fermented material (70 g × 20 of rice) of *Sporormiella minima* (No. 66-3-4-2) was extracted with EtOAc, and the organic solvent was evaporated under vacuum to afford the dry crude extract (30.6 g). Then the crude extract was fractionated by silica gel column chromatography (CC) using cyclohexane–MeOH (100:0 and 0:100, v/v) to afford cyclohexane fraction (C, 17.6 g) and methanol fraction (W, 12.1 g). Methanol fraction was successively subjected to repeated column chromatography over ODS and reversed-phase HPLC (RPHPLC) to obtain compounds **1**–**6**.



1 **2** R = H **3** R = $COCH_3$

Fig. 1 Chemical structures of **1** □ **3**

Sporormiellin A (**1**) was established as $C_{16}H_{14}O_7$ by HRESIMS, with 10 degrees of unsaturation. Analyses of its ^1H, ^{13}C and HSQC data (Table 1) revealed one exchangeable proton (δ_H 12.50), two methyl groups (including one methoxyl), one oxygenated methylene, two sp^3 methines (including one oxygenated methine), one sp^3 oxygenated quaternary carbon, eight olefinic/aromatic carbons (including three protonated), one ester carbonyl (δ_C 169.5), and one α,β-conjugated carbonyl (δ_C 179.5). The ^1H-^1H COSY experiment revealed two isolated spin-systems (C-1**–**C-2**–**C-3/C-15 and C-7**–**C-8**–**C-9) as shown in Fig. 2. The HMBC correlations from the exchangeable proton (δ_H 12.50) to C-9/C-10/C-11, from H-7 (δ_H 6.87) to C-6/C-9/C-11, from H-8 (δ_H 7.47) to C-6/C-10, and from H-9 (δ_H 6.84) to C-7/C-10/C-11 indicated the existence of a 1,2,3-trisubstituted benzene ring in **1**. Furthermore, the weak $^4J_{CH}$ HMBC correlations from H-7/H-9 to C-12 suggested the connection of the α,β-conjugated carbonyl (δ_C 179.5, C-12) to C-11. The methoxyl connected to C-5 based on the HMBC correlation from H_3-16 (δ_H 3.90) to C-5. Combined with the ^1H-^1H COSY analysis, the HMBC correlations from H-3 (δ_H 5.09) to C-1/C-4/C-5/C-15, from H_2-15 (δ_H 3.96, 3.91) to C-1/C-3/C-4, and from H_3-1 (δ_H 1.20) to C-2/C-3/C-15 revealed the existence of a methyl 4-methyltetrahydrofuran-2-carboxylate moiety in **1**. Considering the chemical shifts of C-13 (δ_C 94.6) and C-14 (δ_C 170.1) (β-oxygenated enone system[12]) and the unsaturation requirement for **1**, the planar structure of **1** was established (Fig. 2), and the assignments of all proton and carbon resonances were shown in Table 1.

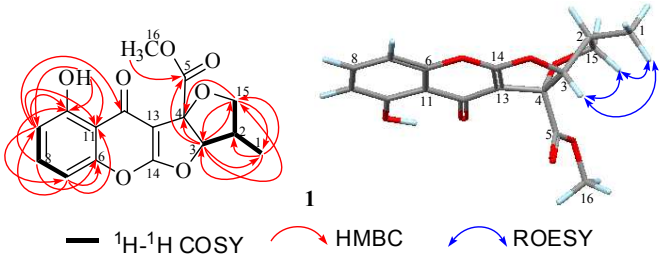


Fig. **2** Key 2D NMR correlations of **1.**

Table 1 1D and 2D NMR data of **1** in $CDCl_3$ (δ in ppm, J in Hz)

No.	δ_C	δ_H	^{1}H-^{1}H COSY	HMBC	ROESY
1	15.6	1.20, d (7.3)	2	2, 3, 15	3, 15$_b$
2	39.9	2.64, m	1, 3, 15$_a$,15$_b$		
3	97.3	5.09, d (0.9)	2	1, 4, 5, 15	1, 15$_b$
4	89.5				
5	169.5				
6	153.8				
7	106.9	6.87, dd (8.3, 0.6)	8, 9	6, 9, 11, 12	
8	134.3	7.47, t (8.3)	7, 9	6, 10	
9	113.4	6.84, dd (8.3, 0.6)	8, 7	7, 10, 11, 12	
10	161.4				
11	109.5				
12	179.5				
13	94.6				
14	170.1				
15	73.0	3.96, dd (9.5, 5.0), a	2	1, 3, 4	
		3.91, dd (9.5, 2.8), b	2	1, 3, 4	1, 3
16	53.5	3.90, s		5	
10-OH		12.50, s		9, 10, 11	

In the ROESY experiment, the observed correlations between H_3-1 and H-15$_b$, between H_3-1 and H-3, and between H-3 and H-15$_b$ demonstrated that H_3-1, H-3 and H-15$_b$ are on the same face of the tetrahydrofuran ring (Fig. 2). The single-crystal X-ray crystallography (Fig. 3) of **1** confirmed the above deduction, and assigned the absolute configuration of **1** as 2S, 3R, 4R. In addition, the conformational analysis for a pair of enantiomers ((2S, 3R, 4R)-**1** and (2R, 3S, 4S)-**1**) was carried out with OMEGA version 2.3 *via* the MMFF94s force field, which provided five lowest energy conformers differing in the dihedral angles of C-1–C-2–C-15–H-15$_a$ and C-13–C-4–C-5–O (0–5 kcal mol^{-1}). The lowest energy conformers (Fig. 4) were submitted to the ECD calculation at [B3P86/6-311++G (2d, p)] level, and the predicted ECD curve of (2S, 3R, 4R)-**1** was similar to the experimental one (Fig. 5, the details see ESI†), which was consistent with the deduction from the X-ray crystallography analysis. Therefore, the absolute configuration of **1** was established as 2S, 3R, 4R.

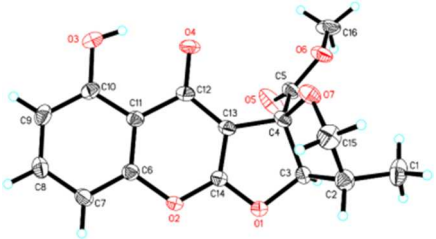


Fig. **3** X-ray structure of **1**

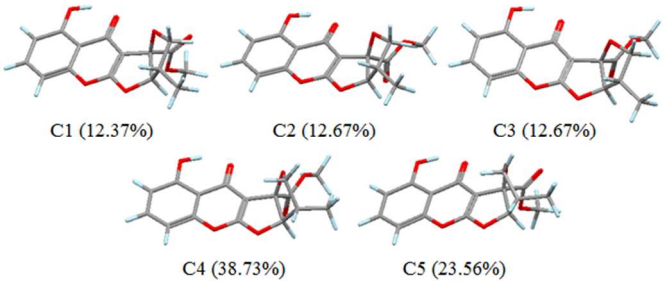


Fig. **4** Most stable conformers of (2S, 3R, 4R)-**1** (the relative populations are in parentheses)

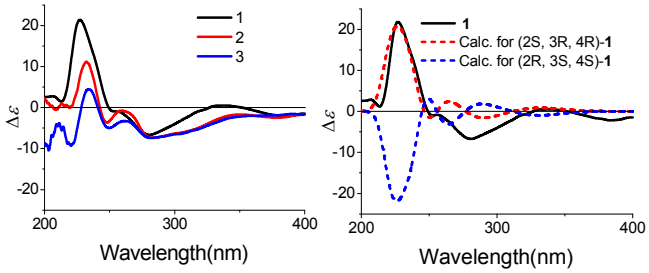


Fig. **5** Experimental ECD spectra of **1**–**3** and calculated ECD spectra of (2S, 3R, 4R)-**1** and (2R, 3S, 4S)-**1**.

The molecular formula of sporormiellin B (**2**) (a pale brown oil) was $C_{16}H_{16}O_8$ (9 degrees of unsaturation) as established by HRESIMS (*m/z* 359.0472, $[M + Na]^+$), which was one degree of unsaturation less than **1**. In addition, the molecular weight was 18 atomic mass units more than **1**, which indicated that one ether ring in **1** should be cleaved by H_2O. Comparative analyses of the ^{1}H and ^{13}C NMR data (in $CDCl_3$, Table 2) with those of **1** revealed that **1** and **2** should have a similar furochromenone skeleton, and differ in the tetrahydrofuran ring moiety, which was confirmed by the analyses of ^{1}H-^{1}H COSY and HMBC. Furthermore, all exchangeable proton signals were detected using DMSO-d_6 in NMR spectrum (the details see ESI†). Therefore, the deduction was confirmed by the observations of the exchangeable proton signal for 15-OH (δ_H 4.78) in the ^{1}H NMR spectrum (in DMSO-d_6) and the key HMBC correlations (in DMSO-d_6) from the exchangeable proton 4-OH (δ_H 6.30) to C-4/C-5/C-13. Therefore, the planar structure of **2** was established as shown in Fig. 6.

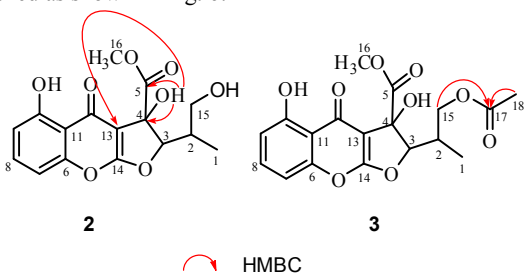


Fig. **6** Key HMBC correlations of **2** and **3.**

In the selective ge-1D NOESY experiment (in DMSO-d_6), an enhancement was observed for H_3-16 in the irridation of H-3, and an enhancement was observed for H-2 in the irridation of 4-OH, which indicated the *cis* relationship of H-3 and C-5. Thus, the relative configuration between C-3 and C-4 was same as that in **1**. In the ECD experiment, the ECD curve of **2** was similar to that of **1** (Fig. 5), which indicated that the absolute configurations of C-3 and C-4 in **2** should be same as those of **1**.

Furthermore, the derivatization of **2** was carried out. **2** was treated with $SOCl_2$ in $CDCl_3$ for 4 hour at 40 ℃, and then the reaction mixture and **1** were compared by HLPC (Fig. 7) (the details see

ESI†), which displayed that the peak of the major reaction product prepared from **2** was identical to the **1** isolated from fungal broth. The derivatization indicated that the configurations of C-2, C-3 and C-4 in **2** were the same as those in **1**. Therefore, the absolute configuration of **2** was assigned as 2S, 3R, 4R.

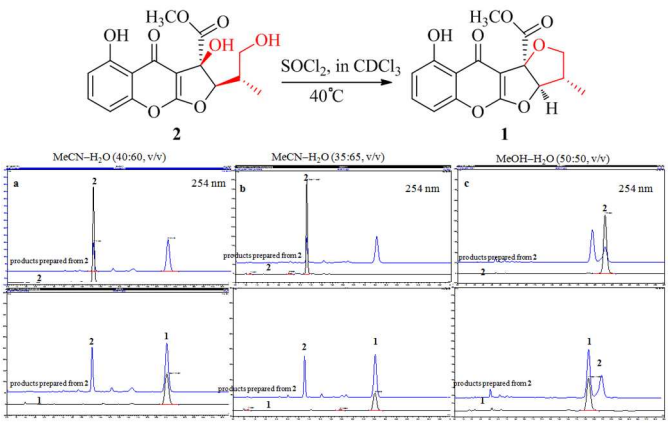


Fig. 7 The products prepared from **2**, **2** and **1** were compared by HLPC with three eluting systems (**a**: MeCN–H_2O (40:60, v/v); **b**: MeCN–H_2O (35:65, v/v); **c**: MeOH–H_2O (50:50, v/v))

Sporormiellin C (**3**) was elucidated as $C_{18}H_{18}O_9$ (10 degrees of unsaturation) by the HRESIMS (*m/z* 401.0844 $[M + Na]^+$). The molecular weight of **3** was 42 atomic mass units more than **2**, which indicated that **3** may be an acetylated derivative of **2**. Except for the signals for one ester carbonyl (δ_C 170.5) and one methyl (δ_C 20.7/δ_H 2.01), the NMR data (in $CDCl_3$) of **3** were similar to those of **2**, which indicated that **3** should also have a furochromenone skeleton. The key HMBC correlations (in DMSO-d_6, for details see ESI†) from H_2-15 (δ_H 4.16, 4.07) to the additional ester carbonyl (δ_C 170.2, C-17) and from the additional methyl protons (δ_H 2.01, H_3-18) to C-17 indicated the existence of the acetyl group and the acetylation at C-15 (Fig. 6).

Table 2 NMR data of **2** and **3** in $CDCl_3$ (δ in ppm, *J* in Hz)

	2		**3**	
No.	δ_C	δ_H	δ_C	δ_H
1	13.4	1.18, d (6.8)	13.4	1.23, d (6.9)
2	34.1	2.61, m	32.1	2.66, m
3	94.4	5.09, d (9.4)	92.4	5.03, d (8.4)
4	78.8		78.5	
5	172.6		172.3	
6	153.8		153.9	
7	107.0	6.87, d (8.3)	107.0	6.88, dd (8.3, 0.6)
8	134.2	7.45, t (8.3)	134.3	7.47, t (8.3)
9	113.2	6.81, d (8.3)	113.3	6.83, dd (8.3, 0.6)
10	161.3		161.4	
11	109.5		109.6	
12	179.5		179.3	
13	98.3		98.3	
14	169.8		169.9	
15	64.8	3.53, dd (10.2, 7.7), a	65.6	4.16, dd (11.5, 4.5), a
		3.78, dd (10.2, 3.5), b		4.07, dd (11.5, 5.6), b
16	54.0	3.90, s	54.3	3.92, s
17			170.5	
18			20.7	2.07, s
10-OH		12.46, s		12.38, s

In the selective ge-1D NOESY experiment (in DMSO-d_6), an enhancement was observed for H_3-16 in the irridation of H-3, which indicated the *cis* relationship of H-3 and C-5. In the ECD experiment, the ECD curve of **3** was similar to that of **2** (Fig. 5), which suggested that the absolute configurations of C-3 and C-4 in **3** should be the same as those of **2**.

Since **2** and **3** coexist in *Sporormiella minima* (No. 66-3-4-2), the configurations of C-2, C-3 and C-4 in **3** should be the same as those in **2**. To confirm the deduction, the derivatization of **3** was carried out. The products prepared from **3** and **2** were compared by HLPC (Fig. 8, the details see ESI†), which displayed that the peak of the major product prepared from **3** was identical to the **2** isolated from fungal broth. Therefore, the absolute configuration of **3** was assigned as 2S, 3R, 4R, which was the same as that of **2**.

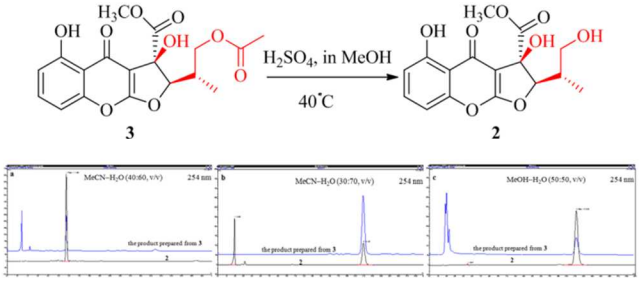
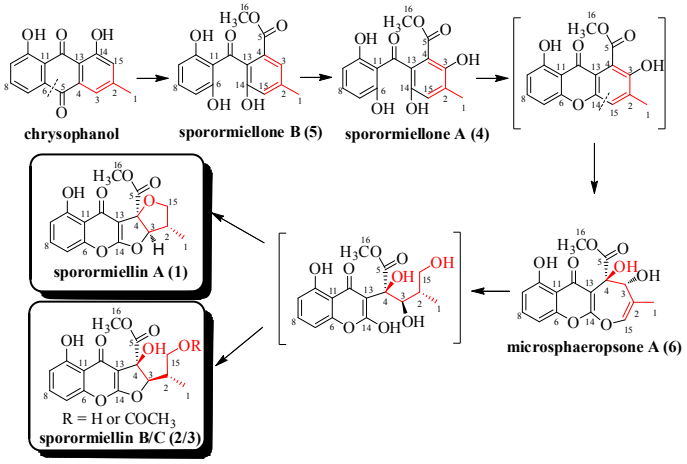


Fig. 8 The products prepared from **3** and **2** were compared by HLPC with three eluting systems (**a**: MeCN–H_2O (40:60, v/v); **b**: MeCN–H_2O (30:70, v/v); **c**: MeOH–H_2O (50:50, v/v))

In addition, three biogenetically related compounds, sporormiellones A (**4**), B (**5**), and microsphaeropsone A (**6**)[13] were also obtained from the fermented material of *Sporormiella minima* (No. 66-3-4-2) (Scheme 1). Among them, sporormiellone A (**4**) is a new benzophenone, whose structure was established by the analysis of 1D and 2D NMR (the details see ESI†). The NMR data of **5** (a known benzophenone) was firstly reported (the details see ESI†), and **5** was named as sporormiellone B.

In summary, sporormiellin A (**1**) is a new member of the furochromenone class of metabolites, possessing an unprecedented tetrahydrofuran-fused tetracyclic skeleton core (6/6/5/5). On the basis of the previous biosynthetic studies of the xanthones from fungi[8, 13] and the structures of obtained compounds (**1**–**6**) by us, sporormiellins A (**1**)–C (**3**) could originate from chrysophanol by oxidative cleavage, ring-expansion, and dehydration/cyclization steps as illustrated in the hypothetical biosynthetic pathway (Scheme 1).



Scheme 1 Plausible biogenetic pathway of **1**–**3**.

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Notes and references

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§ Crystal data for sporormiellin A (**1**): Data were collected using a Sapphire CCD with a graphite monochromated Cu Kα radiation, λ = 1.54178 Å at 173.0 (3) K. Crystal data: $C_{16}H_{14}O_7$, M = 318.27, orthorhombic, space group P212121; unit cell dimensions were determined to be a = 7.2831(2) Å, b = 12.4744(3)Å, c = 16.0184(4) Å, α = 90.00 °, β = 90.00 °, γ = 90.00 °, V = 1455.31(6) Å^3, Z = 4, Dx = 1.453 g/cm^3, F (000) = 664, μ (Cu K_α) = 0.983 mm^{-1}. 11414 reflections were collected until θ_{max} = 62.70°, in which independent unique 2189 reflections were observed [$F^2 > 4\sigma$ (F^2)]. The final refinement gave R = 0.0363, R_W = 0.0991, S = 1.064, and Flack = -0.1(2). Crystal data of **1** was deposited in the Cambridge Crystallographic Data Centre (CCDC 990532).

† Electronic Supplementary Information (ESI) available: the general experimental procedure, fungus material, extraction and isolation, spectroscopic data of **1**–**5**, single-crystal X-ray data of **1**, quantum chemical ECD calculations of **1**, derivatizations of **2** and **3**, and NMR spectra of compounds **1**–**5**. See DOI: 10.1039/c000000x/

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Colour graphic