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Two pairs of enantiomeric α -pyrone dimers from the endophytic fungus *Phoma* sp. YN02-P-3[†]

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(±) Phomones A (1) and B (2), two pairs of novel enantiomeric α -pyrone dimers from the endophytic fungus *Phoma* sp. YN02-P-3 are reported. Compounds 1 and 2 are the first examples of 6- α,β -unsaturated ester-2-pyrone dimers, and compound 1 possesses a novel 6/4/5/6 tetracyclic ring system. Their structures and stereochemistry were determined by the analysis of extensive spectroscopic data, ECD calculations and single-crystal X-ray diffraction data.

[2 + 2] Cycloaddition reactions that construct two new C–C bonds and establish up to four new stereogenic centers in a single step^{1–3} are widely used in the synthesis of natural^{4,5} and bioactive products and have been utilized to synthesize many kinds of important compounds.⁶ The course of the addition reaction and the resulting regioselectivities have remained a topic of great interest in this area.^{7,8} α -Pyrone is a simple heterocyclic dienone system,⁹ which is frequently used as the substrate of intramolecular photochemical reactions, in order to investigate the intermolecular cycloaddition reactivity.^{10–12}

During our continuing search for novel bioactive secondary metabolites from endophytic fungi, *Phoma* sp. was obtained from the sample collected in the plant *Sumbaviopsis* J. J. Smith from Yunnan Province, China. Previous study of this fungus resulted in the isolation of six novel compounds phomeketale A–F,¹³ one novel 3,4-dihydronaphthalen-1(2*H*)-one with spirobutyrolactone and a new isocoumarin.¹⁴ Further investigation led to the discovery of two pairs of novel enantiomeric α -pyrone dimers, (±) phomones A (1) and B (2), and a known compound rosellin (3).¹⁵ Phomones A and B are the first examples of 6- α,β -unsaturated ester-2-pyrone dimers *via* intermolecular unsymmetrical [2 + 2] cycloaddition.^{16,17} Their structures and stereochemistry were elucidated on the basis of the spectral data, single-crystal X-ray diffraction, and ECD analysis. Interestingly, it was found that phomone B (2) slowly transformed to phomone A (1) in MeOH over one month. The effect of H₂O, pH- and temperature-dependent transformation between

compounds 1 and 2, as well as the structural elucidation, postulated biogenetic origin and biological evaluation of these metabolites are reported herein.

(±)-Phomone A (1a/1b) was initially obtained as colorless block crystals. Its molecular formula was established to be C₂₄H₂₈O₁₄ (eleven degrees of unsaturation) on the basis of HRESIMS at *m/z* 563.1352 [M + Na]⁺ (calcd 563.1371). Inspection of ¹H and ¹³C NMR spectra (Table 1) indicated one 4-oxy- α -pyrone ring (δ_C 163.3, 110.0, 168.1, 116.3 and 156.3), one 4-oxy- α -dihydropyrone ring (δ_C 163.2, 107.4, 164.9, 53.1 and 82.6), four methoxyl groups (δ_H 3.54, δ_C 57.9; δ_H 3.65, δ_C 51.6; δ_H 4.03, δ_C 62.1 and δ_H 3.78, δ_C 51.7), five methylene groups (δ_H 4.28, δ_C 52.5; δ_H 4.12/4.29, δ_C 74.4; δ_H 4.35, δ_C 52.8; δ_H 2.53/2.85, δ_C 33.9 and δ_H 4.43/4.33, δ_C 52.2), three methine groups (δ_H 4.62, δ_C 79.8; δ_H 4.26, δ_C 40.8 and δ_H 3.92, δ_C 47.2) and two ester carbonyls (δ_C 170.5 and δ_C 169.2). The HMBC spectrum (Fig. 2) corroborated the presence of the 4-oxy- α -pyrone ring moiety based on correlations from methylene H₂-10' (δ_H 4.35) to C-2' (δ_C 163.3), C-3' (δ_C 110.0) and C-4' (δ_C 168.1) and from H₂-12' (δ_H 4.43/4.33) to C-4', C-5' (δ_C 116.3) and C-6' (δ_C 156.3), and due to the other HMBC correlations, the gross structure of 1 could not be established unambiguously. Fortunately, a crystal suitable for X-ray crystallographic study (CCDC 1504985) was obtained upon slow evaporation of MeOH by keeping the sample at room temperature for one month. The final refinement on the Cu K α data resulted the crystal of 1 had a *p*2₁/*c* space group, indicating a racemic nature, which was in accordance with the lack of optical activity. Furthermore, the X-ray diffraction analysis (Fig. 3) allowed to unambiguously assign the absolute configurations of the two enantiomers of 1 to be (5*R*, 6*R*, 7*R*, 7'*S*, 8'*S*) and (5*S**, 6*S**, 7*S**, 7'*R**, 8'*R**) as Fig. 1, respectively. Separation by using chiral-phase HPLC yielded 1a ([α]_D²⁰ + 30 (c 0.20 MeOH)) and 1b ([α]_D²⁰ – 39 (c 0.20 MeOH)) in a ratio of 1 : 1, whose absolute configurations were established by comparing the calculated ECD spectra with the experimental spectra (Fig. 4). From the above evidence, the absolute stereochemistry for

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[†] Electronic supplementary information (ESI) available: 1D and 2D NMR, HRESIMS, UV, IR, and ECD spectra of phomones and detailed experimental procedures. CCDC 1504985. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ra26319d



Table 1 ^1H and ^{13}C NMR data of **1** and **2**^a

Position	1		2	
	δ_{C}	δ_{H} (m, J in Hz)	δ_{C}	δ_{H} (m, J in Hz)
2	163.2		166.5	
3	107.4		112.4	
4	164.9		169.0	
5	53.1		58.1	
6	82.6		80.1	
7	79.8	4.62 (dd, 9.6, 3.2)	141.4	7.31 (d, 15.6)
8	33.9	2.53 (dd, 9.6, 16.4), 2.85 (dd, 3.2, 16.4)	124.3	6.39 (d, 15.6)
9	170.5		167.7	
10	52.5	4.28 (s)	55.1	4.56 (s)
11	57.9	3.54 (s)	63.0	4.03 (s)
12	74.4	4.29(d, 9.6), 4.12 (d, 9.6)	62.6	3.90 (d, 11.4), 3.75 (d, 11.4)
13	51.6	3.65 (s)	52.4	3.78 (s)
2'	163.3		166.5	
3'	110.0		111.3	
4'	168.1		170.4	
5'	116.3		117.9	
6'	156.3		158.2	
7'	40.8	4.26 (d, 10.0)	37.2	4.20 (d, 11.4)
8'	47.2	3.92 (d, 10.0)	50.9	4.39 (d, 11.4)
9'	169.2		170.4	
10'	52.8	4.35(s)	55.4	4.56 (s)
11'	62.1	4.08 (s)	63.3	4.20 (s)
12'	52.2	4.43 (d, 12.4), 4.33 (d, 12.4)	54.6	4.55 (d, 12.4), 4.46 (d, 12.4)
13'	51.7	3.67 (s)	52.8	3.64 (s)

^a Measured in DMSO-*d*₆ at 400 MHz for ^1H and 100 MHz for ^{13}C .

1a (5*R*, 6*R*, 7*R*, 7'*S*, 8'*S*) and **1b** (5*S**, 6*S**, 7*S**, 7'*R**, 8'*R**) were unambiguously determined as shown in Fig. 1.

(±)-Phomone B (**2a/2b**) shared the same molecular formula of C₂₄H₂₈O₁₄ with compound **1** based on HRESIMS and ^{13}C NMR data. Detailed comparison of its NMR data (Table 1) with those of compound **1** indicated that the main differentiation between compounds **2** and **1** was the absence of the methene [δ_{H} 2.53 (1H, dd, *J* = 16.4, 9.6 Hz), δ_{H} 2.85 (1H, dd, *J* = 16.4, 3.2 Hz)] and the methine [δ_{H} 4.62 (1H, dd, *J* = 9.6, 3.2 Hz)] and the presence of one trans-double bond signals [δ_{H} 6.39 (1H, d, *J* = 15.6 Hz), δ_{H} 7.31 (1H, d, *J* = 15.6 Hz)] in compound **2**, which suggested that the furan ring might be open loop to the double bond additive and the C-12 primary alcohol. The HMBC spectrum (Fig. 2) corroborated the presence of a dihydropyrone ring from H₂-10 (δ_{H} 4.56) to C-2 (δ_{C} 166.5), C-3 (δ_{C} 112.4) and C-4 (δ_{C} 169.0) and from H₂-12 (δ_{H} 3.75/3.90) to C-4, C-5 (δ_{C} 58.1) and C-6 (δ_{C} 80.1) and an α -pyrone moiety based on correlations from H₂-10' (δ_{H} 4.56) to C-2' (δ_{C} 166.5), C-3' (δ_{C} 111.3) and C-4' (δ_{C} 171.4) and from H₂-12' (δ_{H} 4.55/4.46) to C-4', C-5' (δ_{C} 117.9) and C-6' (δ_{C} 158.2). Further HMBC correlations observed from H-7' (δ_{H} 4.20) to C-5 and C-8' (δ_{C} 50.9) and from H-8' (δ_{H} 4.39) to C-7' (δ_{C} 37.2), C-9' (δ_{C} 170.4), C-6 and C-7 (δ_{C} 141.4) demonstrated two α -pyrone rings should be conjugated through a cyclobutane ring and the additional α,β -unsaturated ester moiety was obviously attached to C-6 due to the confirmation of the HMBC correlations from H-

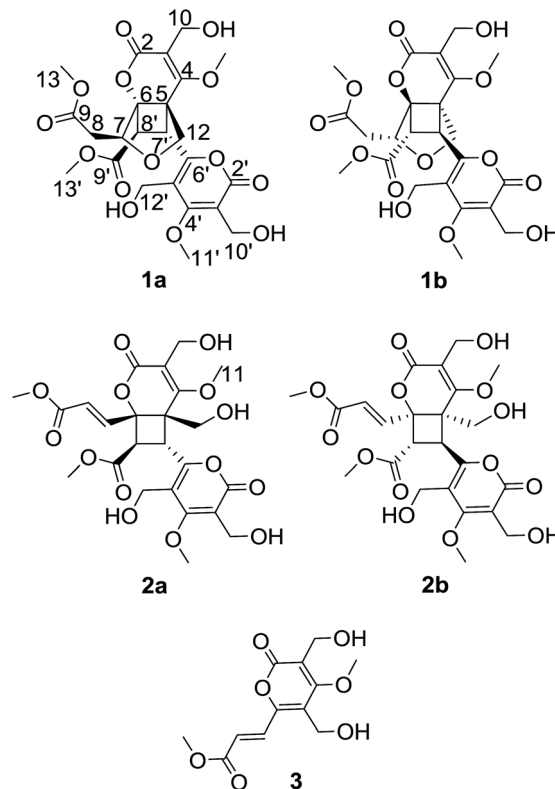


Fig. 1 Structures of compounds **1**–**3**.

7 (δ_{H} 7.31) to C-6 and C-8 (δ_{C} 124.3), and C-9 (δ_{C} 167.7) established the planar structure of compound **2** as shown in Fig. 1.

The NOESY spectrum gave diagnostic correlations of H-7' with H₂-12 and H-7' with H-7, which illustrated H-7', H₂-12, H-7 oriented in the same direction, and analyses of the coupling constants placed H-8' on the opposite side of the cyclobutane ring. Subsequent chiral resolution of compound **2** was performed on a chiral column to yield **2a** and **2b** in a ratio of 1 : 1, which were virtually opposite in terms of their CD curves (Fig. 5). The final assignment of **2a** (5*S*, 6*S*, 7'*R*, 8'*R*) and **2b** (5*R*, 6*R*, 7'*S*, 8'*S*) was made by the comparison of the calculated electronic circular dichroisms (ECD) *via* a quantum method with the experimental data (Fig. 5).

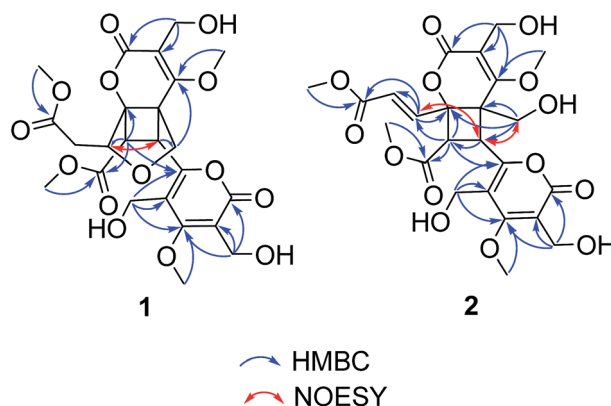


Fig. 2 Key HMBC and Noesy correlations of compounds **1** and **2**.



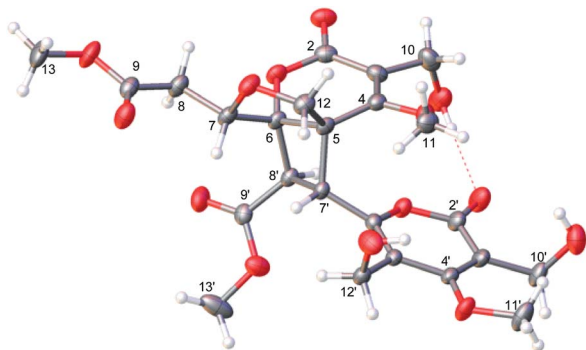


Fig. 3 X-ray crystallographic data for 2.

It was found that compound 2 slowly transformed to compound 1 in MeOH over one month, which indicated that a cyclization reaction was occurring. The effect of H₂O-, pH- and temperature-dependent transformation between phomones A (1) and B (2) were further studied. As shown in Fig. S21,[†] the H₂O-temperature heating experiment suggested that H₂O could promote transformation and the epimerization was quite sensitive to H₂O. The pH-dependent experiment revealed that the transformation was smothered by acid (Fig. S24[†]) and promoted by alkali (Fig. S22[†]). Meanwhile, it was not going to make the alkali promote retransformation successful (Fig. S23[†]). The variable-temperature heating experiment (Fig. S25[†]) revealed that the single compound 2 was stable in anhydrous ethanol solution below 50 °C for 12 hours. Based on the above results, epimerization of 2 and 1 could be induced by H₂O and alkali. The main effect of these transformed observations was H₂O, since H₂O might come into being along with the fermentation. In our study, three pairs of enantiomers, (±) phomones A (1), B (2) and the acetylated products 4a/4b (Scheme 2) were successfully separated by HPLC employing a CHIRALPAK AD-H chiral column (250 × 4.6 mm, 5 μm) and using anhydrous ethanol as the mobile phase at a flow rate of 0.3 mL min⁻¹.

As phomone A (1) and B (2) are 6- α,β -unsaturated ester-2-pyrone dimers, a close biosynthetic relationship could be

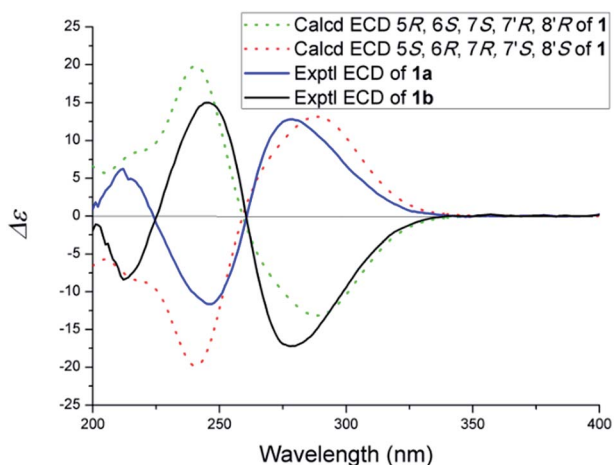


Fig. 4 Experimental and calculated ECD spectra of 1.

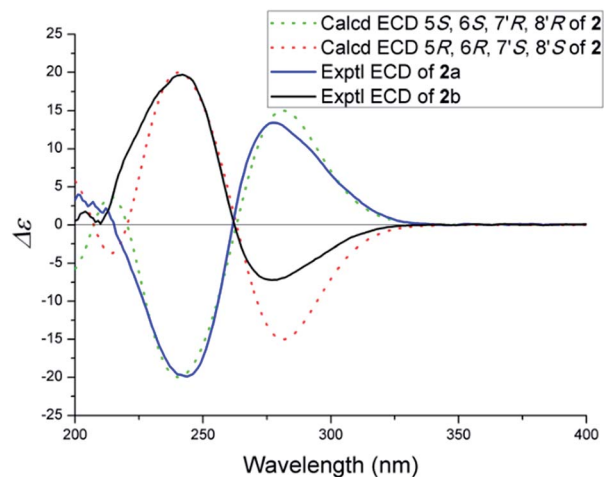
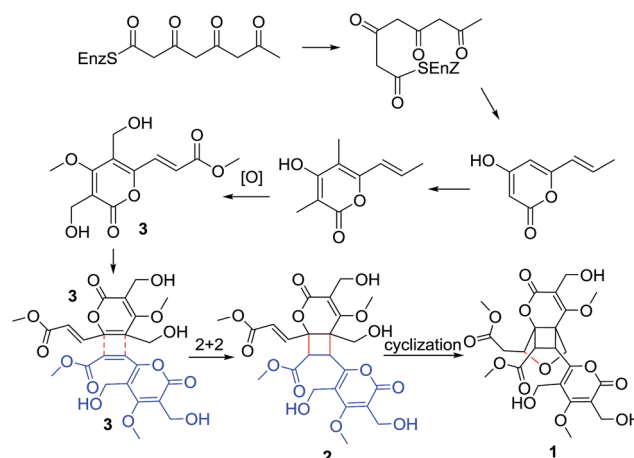
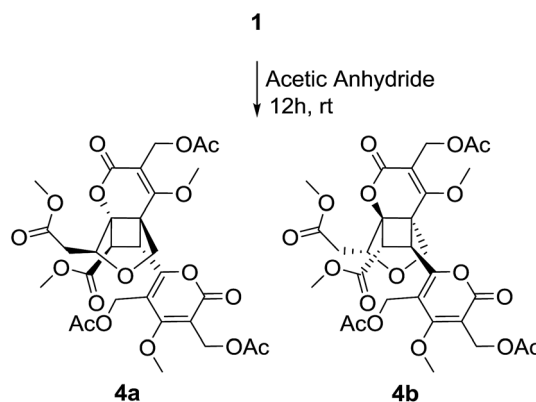


Fig. 5 Experimental and calculated ECD spectra of 2.



Scheme 1 Suggested biosynthetic pathway of 1 and 2.



Scheme 2 Acetylated of phomone A (1).

speculated. Phomone B (2) may be derived through a intermolecular unsymmetrical [2 + 2] cycloaddition reaction of two ethylenic bonds between two rosellisin (3) molecules phomone A (1) could be formed through an intramolecular cyclization reaction of phomone B (2) (Scheme 1).



Compounds **1** and **2** showed no activity ($IC_{50} > 50 \mu M$) when evaluated for their cytotoxic activity against three human cancer cell lines, including human leukemia HL-60, human prostatic carcinoma PC-3 and human colon cancer HCT-116, using 5-fluorouracil as positive control. The acetylated products (**4a/4b**) of compound **1** showed moderate cytotoxic activity against HL-60 cell line with IC_{50} value of 11.05 μM and 14.18 μM , respectively.

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