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The First Naturally Occurring Thiepinols and Thienol from an Endolichenic Fungus *Coniochaeta* sp.

Yinchao Wang,[†,§] Shubin Niu,[†] Shuchun Liu,[†] Liangdong Guo,[†] and Yongsheng Che*[,†,‡]

Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, People's Republic of China, Beijing Institute of Pharmacology & Toxicology, Beijing 100850, People's Republic of China, and Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China

cheys@im.ac.cn

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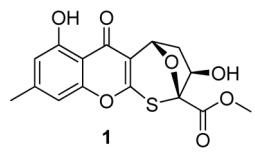
ABSTRACT



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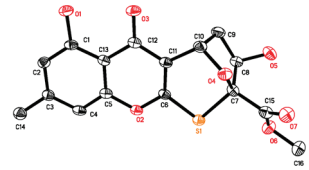

Coniothiepinols A (1) and B (2) and coniothienol A (3), the first naturally occurring thiepinols (1 and 2) and thienol (3), have been isolated from the crude extract of an endolichenic fungus *Coniochaeta* sp. 1 possesses a unique 8-oxa-2-thia-bicyclo[3.2.1]octane skeleton, and its structure was assigned by NMR spectroscopy and X-ray crystallography. 1 showed significant activity against the Gram-positive bacteria, *Enterococcus faecium* and *Enterococcus faecalis*.

Analogous to plant endophytes living in the intercellular spaces of the hosts, endolichenic fungi are microbes that inhabit the thalli of lichens.[1] To date, only a limited number of secondary metabolites have been reported from the endolichenic fungi. Examples include five heptaketides isolated from the *Corynespora* sp.,[2,3] ambuic acid and torreyanic acid derivatives from the *Pestalotiopsis* sp.,[4] and allenyl and alkynyl phenyl ethers from *Neurospora terricola*.[5] Our prior chemical study of the endolichenic fungus *Coniochaeta* sp. also afforded six new xanthone derivatives, such as conioxepinol A (**4**), a cytotoxic oxepinochromenone, and coniofurol A (**5**), a furochromenone.[6] The oxepinochromenones and furochromenones (ring-expanded and ring-contracted xanthones, respectively) are relatively rare, with only a few precedents reported prior to our work.[7−11]

Since the crude extract of *Coniochaeta* sp. also showed antimicrobial activities, and its HPLC chromatogram revealed minor components that could not be identified, the fungus was

* To whom correspondence should be addressed. Tel/Fax: +86 10 82618785.
[†] Institute of Microbiology.
[‡] Beijing Institute of Pharmacology & Toxicology.
[§] Graduate School of Chinese Academy of Sciences.

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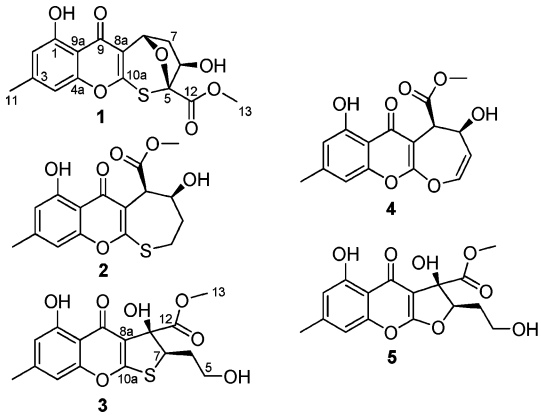
refermented on a larger scale on rice in which the oxepinochromenones and furochromenones were initially isolated. Bioassay-guided separation of an EtOAc extract afforded two thiepinols, coniothiepinols A (**1**) and B (**2**), and a thienol, coniothienol A (**3**). Details of their structure assignment and antimicrobial activities are reported herein.



Coniothiepinol A (**1**) was assigned a molecular formula of $C_{16}H_{14}O_7S$ (10 degrees of unsaturation) by HRESIMS (*m*/*z* 373.0353 $[M + Na]^+$). Its NMR spectra showed resonances for two exchangeable protons, two methyl groups (one methoxy), one methylene, two oxymethines, eight aromatic/olefinic carbons with two protonated, one oxygenated sp^3 quaternary carbon, one carboxylic carbon (δ_C 166.2), and one α,β-unsaturated ketone carbon (δ_C 177.2). The ^1H and ^{13}C NMR data of **1** (Table 1) revealed the same 5-hydroxy-7-methyl-4*H*-chromen-4-one unit as found in **4** and **5**,[6] but the remaining portion was significantly different. The $^1H-^1H$ COSY NMR data of **1** showed the isolated spin-system of C-6−C-8 (including OH-6). HMBC correlations from H_2-7 and H-8 to C-8a, and from H-7b to C-5 led to the connections of C-8 to C-8a and C-5 to C-6, respectively. While that from H-8 to C-5 established an ether linkage between C-5 and C-8. Considering the chemical shifts of C-5 (δ_C 96.9) and C-10a (δ_C 165.4), the only sulfur atom in **1** was attached to both carbons to complete a 4,5-dihydro-2*H*-thiepino[2,3-*b*]chromen-6(3*H*)-one skeleton. An HMBC cross peak from H_3-13 to C-12 connected the C-13 *O*-methyl group to C-12, whereas C-12 was attached to C-5 on the basis of unsaturation requirement, permitting assignment of the plannar structure of **1** as shown.

Finally, **1** was further confirmed by single-crystal X-ray diffraction analysis (Figure 1), and the X-ray data allowed determination of its relative configuration. The presence of a sulfur atom in **1** and the value of the Flack parameter 0.01(10)[12] determined by X-ray analysis also permitted assignment of the absolute configurations of all the chiral centers as 5*R*, 6*R*, and 8*S*.

Table 1. NMR Spectroscopic Data for **1** in Acetone-d_6

position	$\delta_H{}^a$ (*J* in Hz)	$\delta_C{}^b$	HMBC (H → C#)
1		161.5	
2	6.61, s	113.0	1, 4, 9a, 11
3		148.1	
4	6.76, s	107.8	2, 4a, 9, 9a, 11
4a		157.4	
5		96.9	
6	5.06, m	84.6	
7a	2.41, dd (8.0, 3.5)	46.3	6, 8, 8a
7b	2.75, dd (13.5, 8.0)		5, 8a
8	5.79, d (8.0)	73.0	5, 6, 8a, 9, 10a
8a		117.1	
9		177.2	
9a		108.6	
10a		165.4	
11	2.38, s	22.1	2, 3, 4
12		166.2	
13	3.88, s	53.4	12
OH-1	12.29, s		1, 2, 3
OH-6	5.42, d (7.0)		

[a] Recorded at 500 MHz. [b] Recorded at 100 MHz.

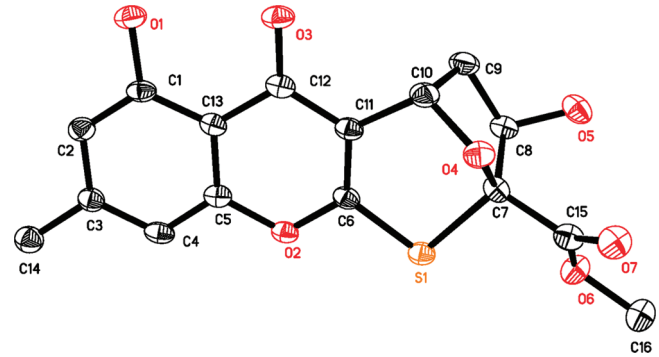


Figure 1. Thermal ellipsoid representation of **1**. (Note: The numbering of structure **1** presented here is consistent with the backbone numbering for **1**. A different numbering system is used for the structural data deposited with the CCDC.)

Compound **2** was given a molecular formula of $C_{16}H_{16}O_6S$ by HRESIMS (*m*/*z* 359.0563 $[M + Na]^+$). Analysis of its NMR spectroscopic data showed structural similarity to **1**, except that the thiepane ring was different. Specifically, the C-8 oxymethine in **1** (δ_H/δ_C 5.79/73.0) was reduced and connected to the methyl formate unit as evidenced by its NMR shifts (δ_H/δ_C 3.93/44.6) and HMBC cross peaks from H-8 and H_3-13 to C-12. While the C-7 methylene in **1** was replaced by an oxymethine (δ_H/δ_C 4.25/66.9), and the C-5 oxygenated sp^3 quaternary carbon was replaced by a methylene (δ_H/δ_C 2.90/26.8), which were supported by relevant $^1H-^1H$ COSY NMR data. Therefore, the gross structure of **2** was determined as depicted.

The relative configuration of **2** was deduced by analogy to **4**.[6] Considering their biogenetic similarity, the C-7 and C-8 stereogenic centers in both compounds presumably have the same configuration, suggesting a *cis* relationship between OH-7 and the methyl formate group, which was partially supported by a NOESY correlation of OH-7 with H_3-13.

The absolute configuration of the C-7 secondary alcohol in **2** was first assigned via the circular dichroism data of an in situ

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formed $[Rh_2(OCOCF_3)_4]$ complex,[13] with the inherent contribution subtracted. Upon addition of $[Rh_2(OCOCF_3)_4]$ to a solution of **2** in CH_2Cl_2, a metal complex with $[Rh_2(OCOCF_3)_4]$ was generated as an auxiliary chromophore. It has been demonstrated that the sign of the E band at ca. 350 nm can be used to correlate the absolute configuration of a secondary alcohol by applying the bulkiness rule.[13,14] In this case, the Rh-complex of **2** showed a positive E band (Figure 2), correlating to the 7*S* absolute

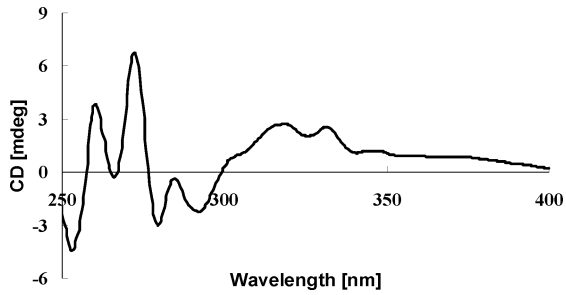
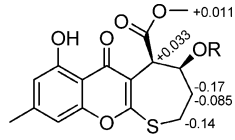


Figure 2. CD spectra of Rh-complex of **2** with the inherent CD spectrum subtracted.

configuration. Considering the possible interference of the carbonyl functionality, the modified Mosher method was also applied.[15,16] Treatment of **2** with (*S*)- and (*R*)-MTPA Cl afforded *R*-(**2a**) and *S*-MTPA (**2b**) monoesters, respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for **2b** and **2a** was calculated to assign the 7*S* configuration (Figure 3). Therefore, the 7*S* and 8*R* absolute configuration



2a R = (*R*)-MTPA ester
2b R = (*S*)-MTPA ester

Figure 3. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ obtained for (*R*)- and (*S*)-MPTA esters **2a** and **2b**, respectively.

was finally assigned for **2** based on the $\Delta\delta$ results summarized in Figure 3.

Compound **3** gave a pseudomolecular ion $[M + Na]^+$ peak at *m/z* 375.0512 by HRESIMS, consistent with the molecular formula $C_{16}H_{16}O_7S$ (nine degrees of C=C unsaturation). Analysis of its NMR spectroscopic data revealed nearly identical structural features to those of **5**, except that the chemical shifts of the C-7 oxymethine in **5** (δ_H/δ_C 5.33/91.6) were different from those of its counterpart in **3** (δ_H/δ_C 4.59/56.7). In addition, the chemical shift of the C-10a sp^2 quaternary carbon in **3** (δ_C 176.2) is also different from that of **5** (δ_C 171.0). Collectively, C-7 and C-10a were both attached to the sulfur atom to establish a 2*H*-thieno[2,3-*b*]chromen-4(3*H*)-one frame, completing the gross structure of **3**.

The relative configuration of **3** was determined on the basis of NOE data. Upon irradiation of H-7 in the NOE experiment, enhancement was observed for H_3-13, suggesting their *cis* relationship, which is consistent with that of **5**. The absolute configuration of the C-8 tertiary alcohol was also first deduced via the CD data of the $[Rh_2(OCOCF_3)_4]$ complex as described for **2** and **5**.[6] The Rh-complex of **3** showed a positive E band near 350 nm (Figure S9, Supporting Information), revealing the 8*S* absolute configuration. Although this assignment could not be verified, the 7*R* and 8*S* absolute configuration was deduced for **3** considering its biogenetic similarity to **5**.

Compounds **1**−**3** were tested for activity against the Gram-positive bacteria, *Enterococcus faecium* (CGMCC 1.2025) and *Enterococcus faecalis* (CGMCC 1.2535), and the plant pathogenic fungus *Fusarium oxysporum* (CGMCC 3.2830) (Table 2). Compound **3** showed significant activity against *E. faecium* and *E. faecalis*, with IC_{50} values of 2.00 and 4.89 μg/mL, repectively, while the positive control ampicillin showed IC_{50} values of 0.51 and 2.61 μg/mL, respectively. Although **1** is less potent than **3** against the bacteria, it displayed modest antifungal activity against the plant pathogen *F. oxysporum*.

Table 2. Antimicrobial Activities of Compounds **1**−**3**

	IC_{50} (μg/mL)		
compd	*E. faecium*	*E. faecalis*	*F. oxysporum*
1	3.93 ± 0.18	11.51 ± 0.45	13.12 ± 0.46
2	>20	>20	>20
3	2.00 ± 0.06	4.89 ± 0.19	>20
ampicillin	0.51 ± 0.014	2.61 ± 0.23	
carbendazim			0.44 ± 0.008

Although *S*-containing natural products have been isolated frequently from fungal sources, coniothiepinols A (**1**) and B (**2**) and coniothienol A (**3**) are the first naturally occurring thiepinols (**1** and **2**) and thienol (**3**), respectively. Compounds **1** and **2** possess the unique 4,5-dihydro-2*H*-thiepino[2,3-*b*]chromen-6(3*H*)-one skeleton, with **1** incoporating the 8-oxa-2-thia-bicyclo[3.2.1]octane partial structure due to the presence of C-5−C-8 ether linkage.

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Supporting Information Available: Experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra of **1**−**3**, CD spectra of **2** and **3**, and X-ray data of **1** (CIF file). This material is available free of charge via the Internet at http://pubs.acs.org.

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