Phytochemistry Letters 27 (2018) 101–104

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

# Sulfurated diketopiperazines from an algicolous isolate of *Trichoderma virens*

Zhen-Zhen Shi[a,b], Feng-Ping Miao[a], Sheng-Tao Fang[a], Xiu-Li Yin[a], Nai-Yun Ji[a,*]

[a] Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

*Keywords:*
*Trichoderma*
Diketopiperazine
Gliotoxin
Sulfide

ABSTRACT

Two new naturally occurring sulfurated diketopiperazines, dehydroxymethylbis(dethio)bis(methylthio)gliotoxin (**1**) and (3*S*,6*R*)-6-(para-hydroxybenzyl)-1,4-dimethyl-3,6-bis(methylthio)piperazine-2,5-dione (**2**), along with three known analogues (**3–5**) were isolated from the culture extract of *Trichoderma virens* Y13-3, obtained from the surface of the marine red alga *Gracilaria vermiculophylla*. The structures and relative configurations of **1** and **2** were determined by extensive 1D/2D NMR, MS, and IR spectroscopic data, and their absolute configurations were established by analysis of ECD spectra aided by quantum chemical calculations. Compounds **1–5** were evaluated for the inhibition of some marine-derived organisms.

## 1. Introduction

Sulfur-containing secondary metabolites have been discovered from various organisms, including terrestrial and marine species (Duan et al., 2007; Li et al., 2013, 2017; Liu et al., 2015; Meng et al., 2013, 2014, 2016; Petkowshi et al., 2018). Among them, marine-derived filamentous fungi have given a number of sulfur-containing compounds with high structural diversity and intriguing bioactivities, which have attracted a great attention for marine natural product research (Ji and Wang, 2016; Meng et al., 2013, 2014, 2016). Gliotoxin with a unique disulfide bridge is a representative fungal toxin that has been obtained from *Trichoderma viride* for the first time (Brian, 1944), and later this compound along with its analogues have also been found in some marine-derived fungi (Chen et al., 2015; Luo et al., 2017; Sun et al., 2012; Wang et al., 2012). In continuation of our efforts to search for the chemical diversity of marine algicolous *Trichoderma* species (Liang et al., 2016; Miao et al., 2012), an epiphytic strain (Y13-3) of *T. virens* obtained from the marine red alga *Gracilaria vermiculophylla* was examined. As a result, two naturally occurring gliotoxin derivatives, dehydroxymethylbis(dethio)bis(methylthio)gliotoxin (**1**) and (3*S*,6*R*)-6-(para-hydroxybenzyl)-1,4-dimethyl-3,6-bis(methylthio)piperazine-2,5-dione (**2**), together with three known analogues, (3*R*,6*R*)-6-(para-hydroxybenzyl)-1,4-dimethyl-3,6-bis(methylthio)piperazine-2,5-dione (**3**) (Hanson and O'Leary, 1981), bis(dethio)bis(methylthio)gliotoxin (**4**) (Lee et al., 2001; Sun et al., 2012), and bis(dethio)bis(methylthio)-12,13-didehydrogliotoxin (**5**) (Forseth et al., 2011; Sun et al., 2012), were isolated and identified from the culture (Fig. 1). Herein, the details of isolation, structure elucidation, and bioactivity of these sulfides are described.

## 2. Results and discussion

Compound **1** was obtained as a colorless oil, and a molecular formula of $C_{14}H_{18}N_2O_3S_2$ was determined by interpretation of HREIMS (*m/z* 326.0760 $[M]^+$, calcd for $C_{14}H_{18}N_2O_3S_2$, 326.0759), requiring seven degrees of unsaturation. The IR spectrum gave absorption bands at 3424 and 1646 $cm^{-1}$, demonstrating the presence of hydroxy and carbonyl groups. The $^1H$ NMR spectrum (in $CDCl_3$, Table 1) in conjunction with HSQC data displayed three methyl singlets, two doublets ascribable to a pair of nonequivalent methylene protons, one singlet and two doublets attributable to three oxygenated or nitrogenated methines, one broad singlet assignable to an exchangeable proton, and one doublet, one doublet of double doublets, and one multiplet due to three olefinic protons. The $^{13}C$ NMR spectrum (Table 1) showed 14 resonances, classified into three methyls, one methylene, six methines, and four nonprotonated carbons by DEPT experiments. A detailed comparison of NMR data with those reported for bis(dethio)bis(methylthio)gliotoxin (**4**) (Lee et al., 2001; Sun et al., 2012) revealed their similarity, except for the lack of signals for a hydroxymethyl group in **1**. Thus, **1** was proposed to be a dehydroxymethyl derivative of **4**, and its planar structure was further verified by the COSY correlations of H-9/H-10/H-11/H-12/H-13 and HMBC correlations from H-3 to C-2 and C-5, H-7 to C-8, C-9, and C-13, from MeS-3 to C-3, from MeN-4 to C-3 and C-5, and from MeS-6 to C-6 (Fig. 2).

The relative configuration of **1** was established to be identical to that of **4** by analysis of NOESY spectra and coupling constants. The NOE correlations of MeS-6 with H-7a and MeS-3 located them on the same face of the molecule, while those of H-13 with H-7b and OH-12

* Corresponding author.
*E-mail address:* nyji@yic.ac.cn (N.-Y. Ji).

https://doi.org/10.1016/j.phytol.2018.07.005
Received 4 April 2018; Received in revised form 30 May 2018; Accepted 10 July 2018

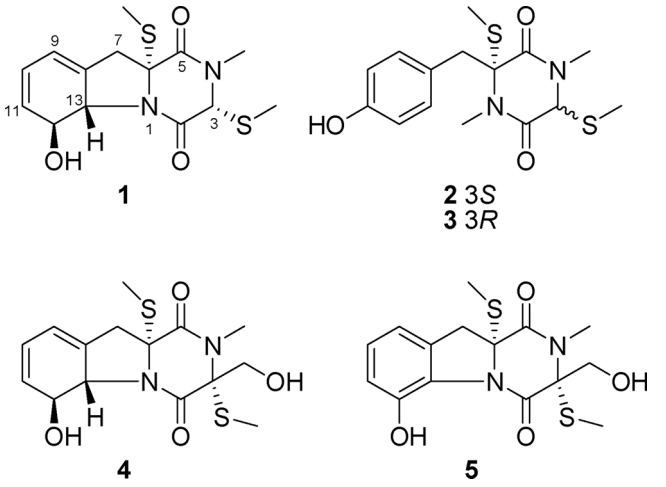


**Fig. 1.** Structures of compounds **1**–**5**.

positioned them on the other face (Fig. 3). The large coupling constants of H-12 and H-13 also suggested their opposite orientation. Regardless of the rotations of OH, MeN, and MeS groups, only one energy-minimized conformer (**1a**) (Fig. 3) optimized at the B3LYP/6–31 G(d) level in MeOH with the integral equation formalism variant (IEF) of the polarizable continuum model (PCM) via Gaussian 09 software (Frisch et al., 2010) was obtained. Its electronic circular dichroism (ECD) spectrum was computed at the same level through the time-dependent density function theory (TD-DFT) method and depicted by SpecDis software with sigma = 0.2 (Bruhn et al., 2011). Based on the comparison of experimental and calculated ECD spectra (Fig. 4), the absolute configuration of **1** was assigned to be 3*R*, 6*R*, 12*S*, and 13*S*. The structure of **1** was previously reported as a synthetic derivative of **4**, but its stereochemistry at C-3 was not determined (Okamoto et al., 1986). It is also worth to mention that $H_2$-7 of the synthetic analogue showed only a broad singlet, rather than two doublets, in the $^1$H NMR spectrum recorded in $CDCl_3$.

Compound **2** was isolated as a colorless oil with a molecular formula of $C_{15}H_{20}N_2O_3S_2$ given by HREIMS (*m/z* 340.0911 $[M]^+$, calcd for $C_{15}H_{20}N_2O_3S_2$, 340.0915), implying seven degrees of unsaturation. The IR absorption peaks at 3422 and 1647 $cm^{-1}$ were slated for hydroxy and carbonyl groups, respectively. The $^1$H NMR spectrum (Table 1) exhibited four methyl singlets, two doublets due to geminal protons of a methylene, one singlet attributable to a deshielded methine, and two

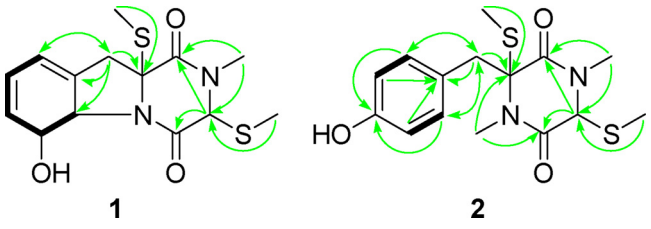


**Fig. 2.** Key COSY (bold lines) and HMBC (arrows) correlations of **1** and **2**.

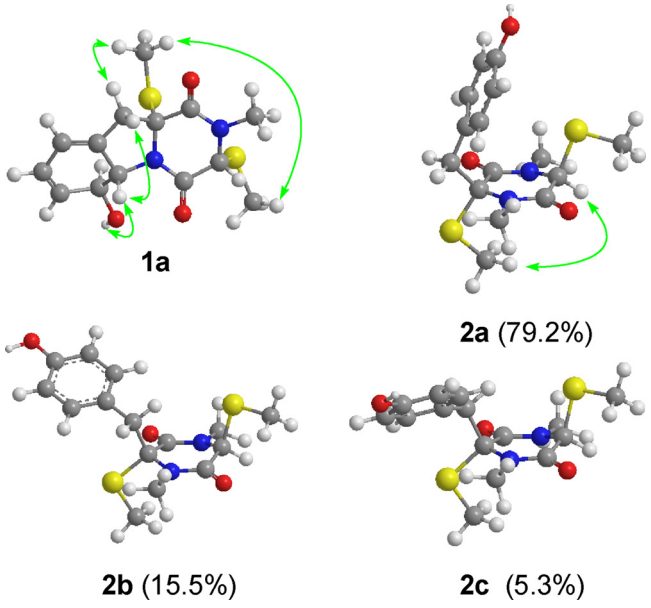


**Fig. 3.** Energy-minimized conformers and NOE correlations of **1** and **2** (Boltzmann populations).

doublets assignable to four aromatic protons. The $^{13}$C NMR spectrum (Table 1) gave only 13 signals, rather than 15 ones as shown in the molecular formula. However, those at $\delta_C$ 115.9 and 131.8 corresponded to two pairs of methines by analysis of HSQC data. The above NMR data, except for the deshielded $^1$H NMR signal for H-3, closely resembled those of (3*R*,6*R*)-6-(para-hydroxybenzyl)-1,4-dimethyl-3,6-bis (methylthio)piperazine-2,5-dione (**3**) (Hanson and O'Leary, 1981).

**Table 1**
$^1$H and $^{13}$C NMR (500/125 MHz) data for **1** and **2** (δ in ppm).

| pos | **1** (in $CDCl_3$) | | **1** (in DMSO-$d_6$) | | **2** (in $CDCl_3$) | |
|---|---|---|---|---|---|---|
| | $\delta_H$ (*J* in Hz) | $\delta_C$, type | $\delta_H$ (*J* in Hz) | $\delta_C$, type | $\delta_H$ (*J* in Hz) | $\delta_C$, type |
| 2 | | 167.3, C | | 167.4, C | | 165.1, C |
| 3 | 4.61, s | 67.8, CH | 5.12, s | 66.1, CH | 4.61, s | 65.9, CH |
| 5 | | 164.6, C | | 164.0, C | | 164.8, C |
| 6 | | 71.9, C | | 71.7, C | | 76.5, C |
| 7a | 3.04, d (16.0) | 38.7, $CH_2$ | 2.99, m | 37.7, $CH_2$ | 3.63, d (14.2) | 40.6, $CH_2$ |
| 7b | 2.94, d (15.9) | | | | 3.06, d (14.2) | |
| 8 | | 131.5, C | | 133.3, C | | 126.3, C |
| 9 | 5.95,m | 120.6, CH | 5.96, m | 119.0, CH | 7.02, d (8.2) | 131.8, CH |
| 10 | 5.89, ddd (9.8, 4.7, 2.4) | 123.1, CH | 5.89, ddd (9.6, 4.7, 2.7) | 123.4, CH | 6.72, d (8.3) | 115.9, CH |
| 11 | 5.76, d (9.8) | 130.5, CH | 5.61, d (9.8) | 130.1, CH | | 155.6, C |
| 12 | 4.90, d (13.1) | 74.4, CH | 4.60, d (13.4) | 73.7, CH | 6.72, d (8.3) | 115.9, CH |
| 13 | 4.84, d (13.3) | 69.3, CH | 4.75, d (13.3) | 68.5, CH | 7.02, d (8.2) | 131.8, CH |
| MeN-1 | | | | | 3.26, s | 30.7, $CH_3$ |
| MeN-4 | 3.11, s | 32.4, $CH_3$ | 2.99, s | 31.4, $CH_3$ | 3.04, s | 33.6, $CH_3$ |
| MeS-3 | 2.45, s | 18.0, $CH_3$ | 2.39, s | 17.1, $CH_3$ | 1.67, s | 14.3, $CH_3$ |
| MeS-6 | 2.20, s | 15.0, $CH_3$ | 2.12, s | 14.2, $CH_3$ | 1.96, s | 12.7, $CH_3$ |
| OH-12 | 5.61, br s | | 5.58, br s | | | |