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Induced production of a new dipeptide with a disulfide bridge by long-term fermentation of marine-derived *Trichoderma* cf. *brevicompactum*

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ABSTRACT

The marine-derived fungus *Trichoderma* sp. TPU199 (cf. *T. brevicompactum*), originally isolated from a Palauan red alga, was found to produce unprecedented epipolythiodiketopiperazines, such as gliovirin and pretrichodermamide A. Long-term static fermentation of the strain induced the production of a new dipeptide, dithioaspergillazine A (**1**), together with aspergillazine A (**2**) and three anthraquinones (**3**–**5**). The structure of **1** was identified as a modified dipeptide possessing a disulfide bridge based on spectroscopic data for **1** and comparisons with those for **2**. On the other hand, long-term agitating fermentation of the strain led to the production of the known bisabolane-type sesquiterpene, (+)-12-hydroxysydonic acid (**6**), which formed the cyclic derivative **7** during HPLC purification under acidic conditions. Compound **1** exhibited cytotoxicity against HCT-15 and Jurkat cells with IC_{50} values of 13 and 1.3 μM, respectively. Compound **2** did not affect the proliferation of these cancer cells up to a concentration of 22 μM.

Microorganisms, such as fungi and actinomycetes, are well-known producers of various bioactive metabolites,[1,2] and fungal metabolites have provided a wide variety of lead compounds for clinical applications.[1,3] Recent studies involving genetic analyses revealed that fungi have a number of biosynthetic genes for secondary metabolites; however, most of them remain dormant under standard fermentation conditions.[4] Therefore, the activation of these silent genes in fungi has attracted significant attention, and several attempts have been made to obtain novel and useful metabolites.[4]

During our investigation on the culture conditions of microorganisms, marine-derived *Trichoderma* sp. TPU199 (cf. *T. brevicompactum*) collected in Palau was found to produce the unique metabolites gliovirin,[5] pretrichodermamide A,[6] and trichodermamide A[7] under ordinary culture conditions as well as the halogenated epidithiodiketopiperazines DC1149B,[8] DC1149R,[8] and iododithiobrevamide[9] in freshwater media supplemented with sodium halides. Moreover, the strain created the new trithio-derivative of DC1149B, chlorotrithiobrevamide, in natural seawater medium with a trace amount of DMSO.[10]

Further culture experiments on strain TPU199 revealed that long-term fermentation induced the production of the new modified dipeptide, dithioaspergillazine A (**1**), under static conditions together with four known compounds: aspergillazine A (**2**),[11] emodine (**3**),[12] pachybasin (**4**),[12] and chrysophanol (**5**).[12] Compound **1** possessed a disulfide bridge instead of the sulfide linkage in **2**. The known bisabolane sesquiterpene, (+)-12-hydroxysydonic acid (**6**),[13] was produced under agitation conditions. Compound **7** was obtained as an artifact from **6** during preparative HPLC using TFA. We herein describe the fermentation, isolation, structural elucidation, and biological activities of the metabolites obtained.

Trichoderma sp. (cf. *T. brevicompactum*) TPU199 was isolated from a red algae collected in Palau and identified from its ITS1 rDNA sequence in a BLAST search.[9]

Strain TPU199 was fermented under four different conditions (A–D),[14] and the HPLC profiles of culture broth extracts were shown in Fig. S1. Gliovirin (**8**), pretrichodermamide A (**9**), and trichodermamide A (**10**) were obtained under static conditions for 2 weeks (A) and under agitation for 1 week (C) in freshwater medium. Static fermentation for 5 weeks (B) in freshwater medium induced the production of compounds **1**–**5**, whereas long-term agitation for 5 weeks (D) led to the production of compound **6** (Fig. S1).

Compounds **1**–**5** were isolated from the culture broth,[15] and compounds **2**–**5** were identified as aspergillazine A (**2**),[11] emodine (**3**),[12] pachybasin (**4**),[12] and chrysophanol (**5**)[12] by comparing their spectroscopic data with reported values (Fig. 1).

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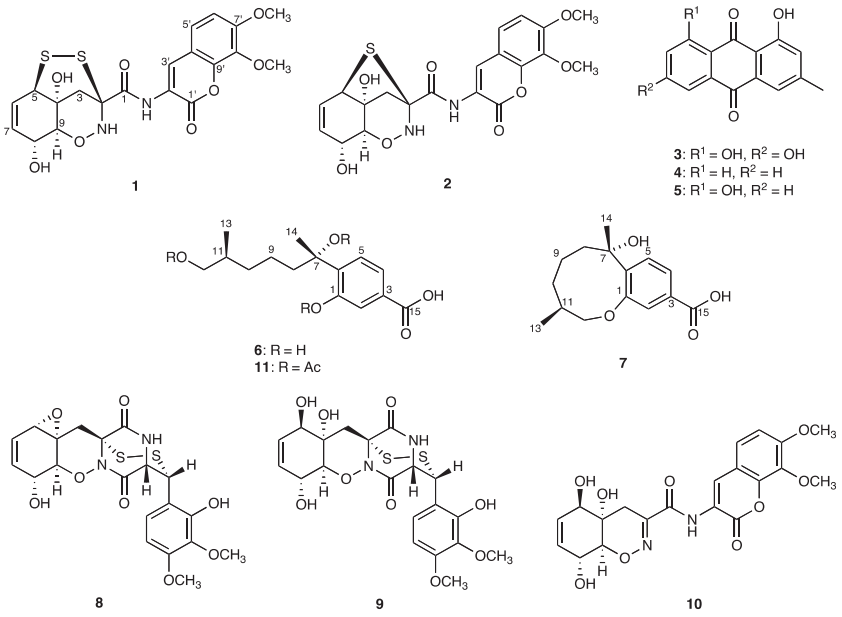

Fig. 1. Structures of compounds **1**–**11**.

Table 1
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for **1** and **2** (in CD_3OD).

C#	**1**		**2**	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	163.5		162.0	
2	73.1		77.3	
3a	41.1	2.33, d (14.0)	50.9	2.37, d (11.7)
3b		3.33, d (14.0)		3.13, d (11.7)
4	65.9		76.2	
5	52.7	4.00, brt (2.9)	47.7	4.13, d (4.9)
6	130.1	6.02 dd (10.1, 3.4)	126.1	5.92, dd (10.0, 4.9)
7	130.7	6.18, dddd (10.1, 5.0, 2.4, 1.0)	128.3	6.05, dd (10.0, 5.0)
8	65	4.44, dd (5.0, 1.9)	65.3	4.34, d (5.0)
9	83.9	4.06, brs	81.8	4.20, brs
1′	159.3		158.3	
2′	121.2		121.4	
3′	118.9	6.99, s	119.2	6.94, s
4′	115.4		116	
5′	129.1	7.05, d (9.2)	129.5	7.00, d (9.0)
6′	105.9	6.64, d (9.2)	105.2	6.58, d (9.0)
7′	156.0		156.1	
7′-OCH_3	56.5	3.87, s	56.4	3.87, s
8′	138.1		138.5	
8′-OCH_3	61.2	3.80, s	61.1	3.80, s
9′	149.1		149.1	

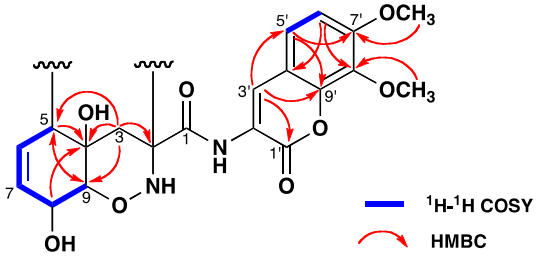

Fig. 2. ^{1}H-^{1}H COSY and key HMBC correlations for compound **1**.

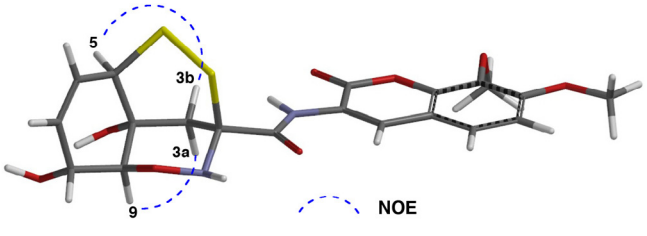

Fig. 3. Key NOESY correlations on the energy-minimized conformer of **1**.

The molecular formula of **1** was deduced as $C_{20}H_{20}N_2O_8S_2$ from HRFABMS (*m/z* 481.0738 $[M+H]^+$, Δ −0.1 mmu) and NMR data. The ^{1}H and ^{13}C NMR data (Table 1) and physico-chemical properties (including UV and IR spectra) of **1** were very similar to those of aspergillazine A (**2**).[11] The molecular weight (formula) of **1** was 32 Da (S) larger than that of **2**. Therefore, the structure of **1** was presumed to be a dithio-derivative of **2**, and an analysis of 2D