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Short communication

Two new 2,5-diketopiperazines produced by *Streptomyces* sp. SC0581

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ABSTRACT

Two new diketopiperazines, cyclo(L-Phe-L-*N*Me-DOPA) (**2**) and cyclo[L-Phe-L-(NMe-3-(*N*Me-3-*O*-α-L-rhamnopyranosyl)-DOPA] (**3**), along with a known diketopiperazine (**1**), were isolated from the cultures of *Streptomyces* sp. SC0581. Their structures were elucidated by extensive spectroscopic analysis, single-crystal X-ray crystallographic analysis, and chemical correlation. Compounds **1** − **3** exhibited more potent ABTS radical cation scavenging activity (IC_{50} values: 3.7 − 14.6 μM) than L-ascorbic acid (IC_{50}: 17.7 μM). Compounds **2** and **3** also showed remarkable DPPH radical scavenging activity.

1. Introduction

2,5-Diketopiperazines, cyclic dipeptides produced by condensation of two α-amino acids, have been found in a variety of natural resources including bacteria, fungi, marine sponge, plants, and mammals (Huang et al., 2010, 2014). They possess a series of intriguing chemical properties, such as resistance to proteolysis, rigid backbone, constrained conformation, stereochemistry controlled at up to four positions, and 2 hydrogen bond donor and 2 hydrogen bond acceptor sites important for binding to various biological targets (Borthwick, 2012). In addition, they have been reported to exhibit diverse biological activities including antibacterial, antifungal, antiviral activities, cytotoxicity, and anti-inflammatory (Martins and Carvalho, 2007). Owing to these chemical and biological properties, 2,5-diketopiperazines have attracted much attention of researchers in synthetic chemistry, medicinal chemistry, and pharmacology. In the course of our searching for novel bioactive metabolites from soil-derived actinomycetes (Feng et al., 2007), two new 2,5-diketopiperazines (**2** and **3**), together with the known diketopiperazine cyclo(L-Phe-L-*N*Me-Tyr) (**1**), were isolated from the cultures of *Streptomyces* sp. SC0581. Herein, we report the isolation, structure elucidation, and antioxidant activity of these compounds.

2. Results and discussion

The known compound cyclo(L-Phe-L-*N*Me-Tyr) (**1**) was identified by comparison of its spectroscopic data and specific rotation with those reported (Liu et al., 2007). In the present study, colorless crystals (mp 160 − 162 °C) of this compound were obtained in MeOH and subjected to X-ray diffraction analysis for the first time, with which its complete structure (Fig. 2), including the absolute configuration, was confirmed (Fig. 1).

Compound **2** was obtained as a white powder. Its (−)-HRESIMS presented a pronounced ion peak at *m/z* 339.1339 $[M - H]^-$, corresponding to a molecular formula of $C_{19}H_{20}N_2O_4$, with one more oxygen atom than that of **1**. Its ^1H NMR and ^{13}C NMR data were closely similar to those of **1**, except that the proton and carbon resonances for the *p*-hydroxyphenyl group in the spectra of **1** were replaced by those indicating the presence of a 3,4-dihydroxyphenyl group [δ_H 6.32 (H, dd, J = 8.0, 2.1 Hz), δ_H 6.67 (1H, d, J = 8.0 Hz), δ_H 6.51 (H, d, J = 2.1 Hz); δ_C 116.8 (CH), 118.5 (CH), 121.8 (CH), 128.2 (C), 145.7 (C), and 146.6 (C)] in **2**, suggesting a 2,5-diketopiperazine composed of a phenylalanine and an *N*Me-DOPA. The planar structure of **2** was supported by 2D NMR data (Fig. 3). With regard to the stereochemistry, compound **2** showed a negative specific rotation value (−110.0) close to that of **1** (−164.0), allowing assignment of the same absolute configuration (3*S*,6*S*) as **1**. Consequently, **2** was elucidated as cyclo(L-Phe-L-*N*Me-DOPA).

Compound **3**, obtained as a white powder, had a molecular formula of $C_{25}H_{30}N_2O_8$ on the basis of the deprotonated ion peak at *m/z* 485.1935 observed in the (−)-HRESIMS. The ^1H NMR and ^{13}C NMR spectra of **3** were comparable to those of **2** except for the presence of additional signals (δ_H 5.34, 4.12, 3.95, 3.50, 3.83, 1.31; δ_C 101.6, 71.9, 72.2, 73.9, 70.8, 18.0) attributable to a rhamnopyranosyl moiety (Fan

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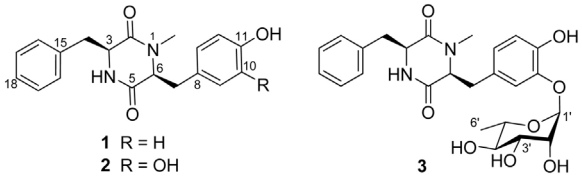

Fig. 1. Structures of compounds **1** − **3**.

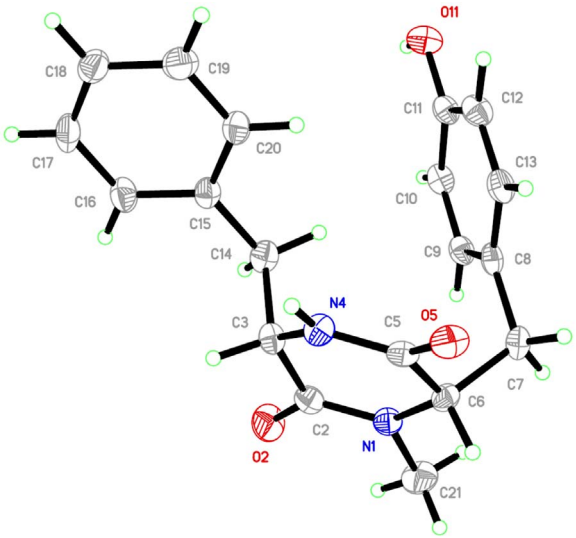

Fig. 2. X-ray structure of **1**.

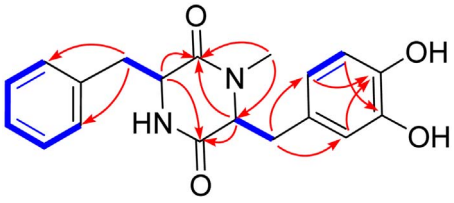

Fig. 3. Key ^{1}H–^{1}H COSY (bold lines) and HMBC (arrows) correlations of **2**.

et al., 2015). The glycosylation site was located at C-10 based on the long-range correlation from H-1′ to C-10 (δ_C 148.3) observed in the HMBC spectrum. The coupling constant (J = 1.7 Hz) of the anomeric proton (H-1′) indicated an α orientation of the glycosidic linkage. The L configuration of the rhamnose moiety was determined using the method described by Tanaka et al. (2007). The 3*S*,6*S* configurations of **3** were assigned by the same negative sign of specific rotation value of **1** and **2**, and on the basis of the co-occurrence of **2** and **3**. Thus, the structure of **3** was defined as cyclo[L-Phe-L-(*N*Me-3-*O*-α-L-rhamnopyranosyl)-DOPA].

Antioxidants play significant role in inhibiting or delaying the oxidation of susceptible cellular substrates and they are important for prevention of many oxidative stress-mediated diseases. Consequently, the antioxidant activity of compounds **1** − **3** was evaluated using ABTS radical cation and DPPH radical scavenging tests and FRAP assay. The results were listed in Table 2. The isolated compounds (**1** − **3**) displayed ABTS radical cation scavenging activity with IC_{50} values ranging from 3.7 to 14.6 μM, which were all superior to that of reference compound, L-ascorbic acid (IC_{50} value: 17.7 μM). In the DPPH radical scavenging assay, compounds **2** and **3** exhibited the activity comparable to that of L-ascorbic acid, but compound **1** was inactive. In FRAP assay, compounds **2** and **3** showed only a mild activity. Tyrosinase inhibitory activity was also evaluated for compounds **1** − **3** using the previously described method (Kim et al., 2016), but none of them was found to be active at the highest test concentration (250 μM).

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds **2** and **3**.

position	**2**[a]		**3**[b]	
	δ_C, type	δ_H, mult. (*J* in Hz)	δ_C, type	δ_H, mult. (*J* in Hz)
2	166.4, C		167.9, C	
3	57.2, CH	3.88, m	58.0, CH	4.01, dd (8.9, 3.8)
5	167.1, C		168.6, C	
6	64.0, CH	4.03, t (4.7)	64.7, CH	4.14, dd (4.6, 5.0)
7a	37.4, CH_2	2.74, d (14.1, 4.7)	37.8, CH_2	2.93, dd (14.3, 4.6)
7b		2.46, d (14.1, 4.7)		2.57, dd (14.3, 5.0)
8	128.2, C		128.5, C	
9	118.5, CH	6.51, d (2.1)	120.9, CH	6.92, d (2.0)
10	146.6, C		148.3, C	
11	145.7, C		146.2, C	
12	116.8, CH	6.67, d (8.0)	117.6, CH	6.86, d (8.0)
13	121.8, CH	6.32, dd (8.0, 2.1)	125.6, CH	6.70, dd (8.1, 2.0)
14a	42.0, CH_2	2.43, dd (13.6, 4.6)	41.8, CH_2	2.73, dd (13.6, 3.8)
14b		1.69, dd (13.6, 8.2)		1.61, dd (13.6, 8.9)
15	138.3, C		137.7, C	
16,20	130.8, CH	7.00, d (7.4)	130.7, CH	7.05, d (7.4)
17,19	129.5, CH	7.27, t (7.4)	129.8, CH	7.32, t (7.4)
18	127.7, CH	7.20, t (7.4)	128.0, CH	7.22, t (7.4)
1-NCH_3	33.7, CH_3	2.81, s	33.9, CH_3	2.93, s
4-NH		7.82, brs		
1′			101.6, CH	5.34, d (1.7)
2′			71.9, CH	4.12, dd (3.5, 1.7)
3′			72.2, CH	3.95, dd (9.5, 3.5)
4′			73.9, CH	3.50, t (9.5)
5′			70.8, CH	3.83, dd (9.5, 6.2)
6′			18.0, CH_3	1.31, d (6.2)

[a] DMSO-d_6 used as solvent.
[b] CD_3OD used as solvent.

Table 2
Antioxidant activity of compounds **1** − **3**.

compound	ABTS (IC_{50}, μM)	DPPH (IC_{50}, μM)	FRAP (mmol/g)
1	3.7 ± 0.13	> 250	0.21 ± 0.002
2	14.6 ± 0.70	37.2 ± 0.96	7.15 ± 0.079
3	7.1 ± 0.83	27.7 ± 0.09	6.87 ± 0.014
L-ascorbic acid	17.7 ± 0.40	36.2 ± 0.18	13.31 ± 0.030

Values represent means ± standard deviation (SD) (n = 4).

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a Perkin-Elmer 343 spectropolarimeter. UV spectra were measured with a Perkin EImer Lambda 650 UV/VIS spectrometer. 1D and 2D NMR experiments were performed on a Bruker Avance III 500 Hz instrument. HR-ESIMS data were recorded on a Bruker maXis Q-TOF mass spectrometer. Preparative HPLC was run on a Shimadzu Shim–packed Pro-ODS column (20 mm × 25 cm). For column chromatography, silica gel 60 (100–200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), YMC ODS (75 μm, YMC Co. Ltd., Kyoto, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) were used. TLC was carried out on HSGF254 silica gel plates (0.2 mm, Yantai Jiangyou silica gel Development Co. Ltd., Yantai, China); spots were visualized by spraying with 10% H_2SO_4 in EtOH followed by heating.

3.2. Biological material and fermentation

The actinomycetes strain *Streptomyces* sp. SC0581 was isolated from a soil sample collected in Dinghu Mountain Biosphere Reserve, Guangdong, China, and authenticated based on morphological characteristics and DNA sequence analysis of the ITS region (GenBank accession no: KX687558). A voucher strain is deposited in the culture