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Three Naphthoquinones from *Streptomyces* sp. XZYN-4

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Abstract: Two new naphthoquinones, named 12-hydroxy-naphthgeranine A (**1**) and isonaphthgeranine C (**2**) together with naphthgeranine A (**3**), were isolated from the cultures of *Stereospermum*'s rhizosphere strain *Streptomyces* sp. XZYN-4. Their structure were established on the basis of 1D, 2D-NMR experiments and HR QTOF MS. The absolute configurations of C-12 (12*R*) in both (**1**) and (**2**) are different from those reported in naphthgeranine family. Compounds **1** and **3** showed moderate activity against *Aspergillus niger* ACCC30005 by disk diffusion test at a dose of 20 μg/disk.

Keywords: *Streptomyces* sp. XZYN-4; naphthoquinone; 12-hydroxy-naphthgeranine A; isonaphthgeranine C.

1. Introduction

In the course of our screening for antifungal strains, an isolate namely XZYN-4 obtained from *Stereospermum*'s rhizosphere, was selected. Further screening has resulted in the isolation of three compounds. This paper describes the isolation and characterization of compounds **1 - 3** and their antimicrobial effects against *Candida albicans* ATCC10231, *Aspergillus niger* ACCC30005, and *Escherichi coli* ATCC25922.

2. Materials and Methods

2.1. Microorganism Material

The strain XZYN-4 was identified as *Streptomyces* sp. according to its 16s rDNA sequence (Accession No. HM060198).

2.2. Fermentation and Isolation

The strain was inoculated on a slope of SYP (starch 10%, yeast extract 4%, peptone 2% and agar 1.5%, at pH 7.2) media in a test tube and cultivated for 7 d at 28 °C to afford seed cultures. Solid state fermentation (5 L) was performed on SYP medium for 7 d at 28 °C.

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The strain XZYN-4 was fermented and was extracted three times with equal volume of EtOAc/MeOH/AcOH 80 : 15 : 5 (*v/v/v*) at room temperature. The organic solutions were collected by filtration and removed under vacuum at 40 °C. The concentration was extracted with EtOAc and water (1:1) for three times and the EtOAc solution was collected under vacuum at 40 °C to obtain 1.0 g extract.

The extract (1.0 g) was subjected to MPLC (70 g RP-18 silica gel; H_2O, MeOH/H_2O 30%, 50%, 70%, MeOH, 1 l respectively) to afford 3 fractions: Fr.A - C. Fr.A (32 mg) obtained from 70% MeOH was subjected to *Sephadex LH-20* (80 g; MeOH and acetone) to yield **3** (1.0 mg).

Fr.B obtained from 50% MeOH (60 mg) was further purified by CC (*Sephadex LH-20,* 40 g, MeOH) to yield Fr.B1 (10 mg), and Fr.B1 was further purified by MPLC (70 g RP-18 silica gel; 60% and 70% MeOH, 400 mL each) to obtain Fr.B1a (5 mg). Fr.B1a was subjected to CC (SiO_2; petroleum ether/ethyl acetate 10:1; 7:1) to afford **2** (3.0 mg).

Fr.C (96 mg) obtained from 50% MeOH was purified by CC (*Sephadex LH-20,* 80 g, MeOH) to yield Fr.C1 (10 mg), and Fr.C1 was further purified by HPLC (Agilent 1200 series, column: 250 × 4.6 mm, 220 nm, MeOH/H_2O 55:45, 1.0 mL/min, t_R=13.0 min) to afford **1** (2.0 mg).

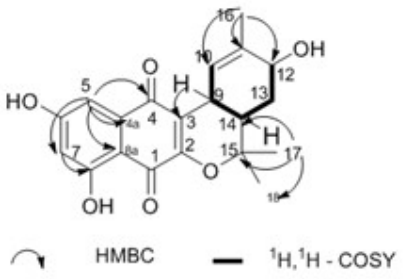

Figure 1. The selected HMBC and ^{1}H, ^{1}H-COSY correlations of **1**

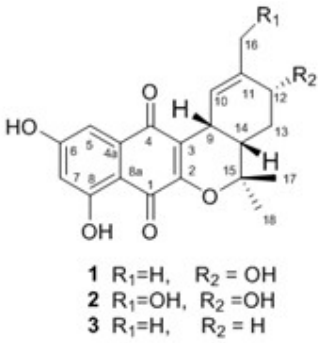

Figure 2. Structures of compounds **1-3**

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as yellow powder. The molecular formula of **1** was determined to be $C_{20}H_{20}O_6Na^+$ by HR-ESI-MS (*m/z* 379.1157 $[M + Na]^+$, calcd for 379.1152). The IR spectrum showed absorption bands for hydroxyls at 3445 cm^{-1} and conjugated aromatic ketone functionalities at 1631, 1457 and 1280 cm^{-1}. The ^{13}C NMR (DEPT) spectrum of **1** showed 20 signals for three Me, one CH_2, six CH, and ten quaternary carbons including two keto carbons at δ 182.7 and 182.9 (Table 1).

The naphthoquinone moiety was deduced from observed HMBC correlations: H-5 to C-8a, C-7, C-4a and C-4; H-7 to C-5 and C-8. Based on the ^{13}C chemical shifts, C-6 and C-8 were assigned to phenolic carbons. Thus, a 1,3-dihydroxy-4,5-disubstituted benzene system was accommodated [1]

A 3,4-disubstituted 1-methylcyclohexene skeleton was deduced according to the ^{1}H-^{1}H COSY and HMBC correlations. The sequence from H-10 to H-12 through H-9, H-14 and H-13 was revealed by ^{1}H-^{1}H COSY spectrum. H-16 showed HMBC correlations to C-10, C-11 and C-12.

The structure of the cyclohexene ring moiety of **1** (Figure.1) was extended by HMBC spectrum analysis; the methyl proton H-17 was coupled to C-14, C-15 and C-18. Likewise, another tertiary methyl proton H-18 was coupled to C-14, C-15 and C-17. Additional two carbons were C-3 and C-2. According to the chemical shifts of C-2 and C-15 and a quasi-molecular ion peak at *m/z* 379.1157 $[M + Na]^+$ provided by HRQTOFMS, C-2 and C-15 had to be connected by an ether bond as shown in Figure 1 [1]

The relative configuration of **1** was determined by analysis of the NOESY spectrum. The presences of NOE correlations between H-12 and H-14 and H-17, as well as between H-9 and H-17 indicated that

the relevant protons are on the same side of the molecule (Figure 1). Therefore, the structure was elucidated as 12(*R*)-hydroxy-naphthgeranine A [1,2].

The structures of the isonaphthgeranine C (**2**) [3,4] and naphthgeranine A (**3)** [1,2] could be determined by comparison of the NMR and mass spectroscopic data with those of reported and **1**.

The NMR data of **2** was very similar to that of **1** (Table 1), except that the CH_3 at δ 18.6 (C-16) in **1** was replaced by an oxygenated methylene at δ 62.3 in **2**, and an additional oxygen atom was further suggested from MS data *m/z* 395.2 $[M + Na]^+$ and *m/z* 411.2 $[M + K]^+$. The presences of NOE correlations between H-12 and H-14, as well as between H-9 and H-14 indicated that the relevant protons are on the same side of the molecule (Figure. 1). Therefore, the configuration at C-12 of **3** is the same as that of **1,** but differ from naphthgeranine C [3.4]. Therefore, the structure was elucidated as isonaphthgeranine C.

Compound **3** (Figure. 2) shows very close correspondence to **1**, in which a CH at δ 68.6 (C-12) disappeared and was replaced by a CH_2 carbon at δ 20.2, and further confirmed by the quasi-molecular ion peak at *m/z* 341.1 $[M + H]^+$. Therefore, the structure was elucidated as naphthgeranine A [3].

Table 1. The NMR data of compounds **1–3.** At 600 and 150 MHz in acetone-d_6, δ in ppm, *J* in Hz.

position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		182.7(C)		182.3(C)		182.7(C)
2		153.0(C)		153.2(C)		152.9(C)
3		123.0(C)		122.4(C)		123.1(C)
4		182.9(C)		183.4(C)		182.9(C)
4a		135.2(C)		134.7(C)		135.0(C)
5	7.04(*d*,2.5)	107.8(CH)	7.00(*d*,2.0)	108.4(CH)	7.04(*d*,2.2)	107.8(CH)
6		165.0(C)		166.0(C)		163.5(C)
7	6.53(*d*,2.3)	106.2(CH)	6.46(*d*,2.1)	106.0(CH)	6.53(*d,*2.3)	106.2(CH)
8		164.0(C)		164.4(C)		165.0(C)
8a		108.0(C)		107.3(C)		108.0(C)
9	3.44*(t*,5.1)	31.5(CH)	3.56(*t,6.0*)	31.1(CH)	3.47(*t*,4.4)	31.1(CH)
10	6.13(*d*,5.4)	121.7(CH)	6.51(*d*,4.8)	123.0(CH)	6.10(*d*,4.4)	120.5(CH)
11		139.6(C)		141.8(C)		135.2(C)
12	4.11(*dd*,5.0,10.2)	68.6(CH)	4.35(*dd*,5.0,10.2)	66.7(CH)	1.29 (*m*)	20.2(CH_2)
13	2.24(*dd*,5.0,12.6);1.26(*t*,12.6)	30.0(CH_2)	2.29(*dd*,5.5;12.0) 1.31(m)	29.9(CH_2)	1.97 (*m*) 1.29 (*m*)	30.0(CH_2)
14	2.04(*ddd*,2.5, 6.1, 13.2)	39.0(CH)	2.06(*ddd*,2.3; 8.3;13.1)	38.8(CH)	1.86 (*m*)	39.4(CH)
15		80.0(C)		80.2(C)		80.0(C)
16	1.73(*s,*3H)	18.6(CH_3)	4.24(*d*,13.4) 4.11(*d*,13.3)	62.3(CH_2)	1.65(*s*, 3H))	22.8(CH_3)
17	1.53(*s,* 3H))	25.1(CH_3)	1.57(*s*,3H)	24.5(CH_3)	1.52(*s*, 3H))	24.9(CH_3)
18	1.34(*s,* 3H))	23.7(CH_3)	1.39(*s*, 3H)	23.2(CH_3)	1.36(*s*, 3H))	24.3(CH_3)

3.2.3.2. Antimicrobial Activity

Our results suggested that **1** and **3** showed moderate Activity against *Aspergillus niger* ACCC30005 with inhibitory zone of 16 mm and 12 mm at 20 μg/disk dose, but no activity against *Escherichia coli* ATCC25922, and *Candida albicans* ATCC10231 was observed. Compound **2** showed no activity against tested microbes.

4. Discussion

From 5 l fermentation extract, three naphthoquinones (**1** - **3**) including two new ones (**1** and **2**) were isolated from *Streptomyces* sp. XZYN-4. Their absolute configurations of C-12 were determined as *R* according to the NOE correlations, which were distinct from the configurations found in naphthgeranine family previously [3]. They all belong to a small group of microbial quinones [3] in which structural

elements derive from the polyketide pathway and from mevalonic acid building blocks as well [5]. These classes of secondary metabolites are more typical for plants and fungi than for *Streptomyces*.

Previous studies suggested that naphthgeranine A (**3**) was isolated as a free radical scavenger [2]; Naphthgeranine C showed certain cytotoxic activity against tested tumor cell lines [3]; Our finding not only provides important new additions to the family of naphthoquinone metabolites, but also reveals moderate activity against *A. niger*.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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