

## ORIGINAL ARTICLE



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## Three New 2-pyranone Derivatives from Mangrove Endophytic Actinomycete Strain *Nocardiosis* sp. A00203

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**Abstract:** Three new 2-pyranone derivatives, namely Norcardiatones A (1), B (2) and C (3), were isolated from the agar cultures of the strain *Nocardiosis* sp. A00203, a mangrove endophytic actinomycete. Their structures were elucidated by spectroscopic and mass-spectrometric analyses, including 1D-, 2D-NMR and HR Q-TOF-MS. Compound 1 showed week cytotoxicity against HeLa cells in MTT assay.

**Keywords:** 2-pyranone derivatives; *Nocardiosis* sp. A00203; spectroscopic analyses.

### 1. Introduction

Mangrove and semi-mangrove plants are mostly distributed in the tropical and subtropical coastal regions of the world. Because of their special ecosystems that straddle the land and the sea, mangrove plants are found to be a rich source of endophytic fungal species [1–2]. More than 200 species of endophytic fungi have been isolated and identified from mangrove plants, being the second largest community of marine fungi. Studies revealed that the endophytic fungi of mangrove can produce many kinds of metabolites with great potential for anti-microbial and anti-tumor medicinal use [3]. Several actinomycetes isolated from root soil of mangrove have been studied, and several antimicrobial and cytotoxic compounds were obtained [4,5]. However, the mangrove ecosystem is a largely unexplored source for actinomycetes with the potential to produce biologically active secondary metabolites. In this paper, three new 2-pyranone derivatives (1–3) were isolated and identified from the mangrove actinomycetes *Nocardiosis* sp. A00203

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## 2. Materials and Methods

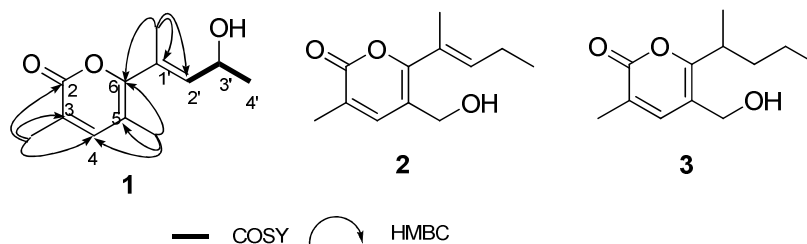
### 2.1. Microorganism Material

The strain A00203 was isolated from the leaves of *Aegiceras corniculatum* collected from Jimei, Fujian Province, China. Both a traditional morphological assessment and 16S rDNA sequencing (GenBank accession number: AJ539401.1) were performed to characterize it as *Nocardiopsis* sp., and named as A00203. A nucleotide-to-nucleotide BLAST query of the NCBI database yielded *Nocardiopsis aegyptica* strain DSM44442 as the closest match to the 16S rDNA of A00203 (98%).

### 2.2 Fermentation and Isolation

The fermentation of *Nocardiopsis* sp. A00203 was performed on YMG (5 L) and YEME (10 L) agar plates for 7 d at 28°C. The cultures of two different media were diced and extracted with EtOAc/MeOH/AcOH (80 : 15 : 5, v/v/v), respectively. The two crude extracts were partitioned between EtOAc and H<sub>2</sub>O (1 : 1) until the EtOAc layer was colorless. The combined organic layers were dried over sodium sulfate (anhydrous) and concentrated *in vacuo* to afford EtOAc extracts 1.38 g (YMG medium) and 2.10 g (YEME medium), respectively. The EtOAc extract (1.38 g) was subjected to MPLC (*RP-18* (145 g), MeOH/H<sub>2</sub>O (1 : 1)) to afford *Fr.1*. *Fr.1* (97 mg) was further subjected to *Sephadex LH-20* column (in MeOH) to afford two fractions *Fr.1.1* – *Fr.1.2*. *Fr.1.2* (27 mg) was separated to *Fr.1.2.1* and *Fr.1.2.2* by MPLC (*RP-18* (30 g), MeOH/H<sub>2</sub>O (9 : 11)). *Fr.1.2.2* was subjected to silica gel chromatography (petroleum ether/acetone 100:1 and 50:1) to yield **1** (2.0 mg).

The EtOAc extract (2.10 g) was subjected to MPLC (*RP-18* (130 g), MeOH/H<sub>2</sub>O (1 : 1)) to afford two fractions *Fr.1* - *Fr.2*. *Fr.2* (80 mg) was subjected to *Sephadex LH-20* column (in MeOH) to afford *Fr.2.1* (18 mg), which was further separated to *Fr.2.1.1* and *Fr.2.1.2* by MPLC (*RP-18* (30 g), MeOH/H<sub>2</sub>O (1 : 1)). *Fr.2.1.2* was subjected to silica gel chromatography (petroleum ether/acetone 100:1 and 80:1) to yield **2/3** (2.0 mg).



**Figure 1.** <sup>1</sup>H-<sup>1</sup>H COSY correlations and the selected HMBC correlations of compound **1** and the structures of compounds **1-3**.

## 3. Results and Discussion

### 3.1. Structure elucidation

Compound **1** was isolated as yellow oil. The molecular formula was determined to be C<sub>12</sub>H<sub>16</sub>O<sub>3</sub> with five degrees of unsaturation in the molecule by HR Q-TOF-MS (*m/z* 231.1001 [*M* + Na]<sup>+</sup>, calcd. 231.0997) and NMR data (Tables 1 and 2). The IR spectra showed the absorptions for OH groups (3285 cm<sup>-1</sup>), and conjugated carbonyl (1726 cm<sup>-1</sup> and 1691 cm<sup>-1</sup>) groups. The <sup>13</sup>C NMR spectrum of **1** displayed twelve signals corresponding to four methyls, three methylenes (one being oxygenated), and

five quaternary carbon atoms, including a carbonyl carbon at  $\delta$ 163.3 (C(2)). The HMBC correlations from H-C(3a) to C(2), C(3) and C(4), from H-C(5a) to C(4), C(5) and C(6), from H-C(1'a) to C(1'), C(2') and C(6), along with  $^1\text{H}$ - $^1\text{H}$  COSY correlations H-C(2') $\leftrightarrow$ H-C(3') $\leftrightarrow$ H-C(4') established the crude structure of **1** (Figure 1). One carbonyl and three double-bond groups can explain four degrees of unsaturation, and the remaining one indicated the linkage of C(2) and C(6) *via* oxygen due to the chemical shift of C(6) ( $\delta$ C) 157.3), which was relatively upfield shifted compared to regular COOH groups. It was consistent with the skeleton of pyranone [6, 7]. The configuration of C=C between C(1') and C(2') was determined to be (*E*) by the NOE correlations H-C(3') $\leftrightarrow$ H-C(1'a) $\leftrightarrow$ H-C(2'). Therefore, compound **1** was determined to be 6-(4-hydroxypent-2*E*-en-2-yl)-3, 5-dimethyl-2H-pyran-2-one, namely norcardiatone A (Figure 1).

Compounds **2/3** were isolated as yellow oil. The IR absorptions indicated the presence of OH groups (3353  $\text{cm}^{-1}$ ), carbonyl (1667  $\text{cm}^{-1}$ ) and unsaturated carbon/carbon double bonds (1633 and 1596  $\text{cm}^{-1}$ ). Analysis of the  $^{13}\text{C}$  NMR spectrum of **2**, twenty four carbon signals for six methyls (at  $\delta$  18.9, 16.5, 16.4, 14.5, 13.9, 13.5), five methylenes (at  $\delta$  60.3, 59.7, 36.7, 21.7, 20.8), one methane (at  $\delta$  34.4), four carbonyl or oxygenated double bond carbons (at  $\delta$ 164.3, 163.8, 163.2, 161.1) and eight unsaturated carbons (at  $\delta$ 141.9, 141.8, 138.7, 126.7, 122.8, 123.5, 114.0, 113.9) were observed (Tables 1 and 2). Careful inspection of the HMBC correlations from six methyls to corresponding carbons and  $^1\text{H}$ - $^1\text{H}$  COSY correlations, two independent structures were deduced to be **2** and **3** as shown (Figure 1). The HR Q-TOF-MS gave quasi molecular ion peaks at  $m/z$  209.1180 [ $M + \text{H}$ ] $^+$  (calcd. 209.1178), 231.0994 [ $M + \text{Na}$ ] $^+$  (calcd. 231.0997), 211.1330 [ $M + \text{H}$ ] $^+$  (calcd. 211.1334), and 233.1150 [ $M + \text{Na}$ ] $^+$  (calcd. 233.1154), which also indicated a mixture **2/3**. The ratio of **2/3** was about 1 : 1 based on the integral of protons in  $^1\text{H}$  NMR. Compared NMR and MS data with those of **1**, compounds **2** and **3** were determined to be 5-(hydroxymethyl)-3-methyl-6-(pent-2*E*-en-2-yl)-2H-pyran-2-one and 5-(hydroxymethyl)-3-methyl-6-(pentan-2-yl)-2H-pyran-2-one, namely norcardiatones B (**2**) and C (**3**).

**Table 1.**  $^1\text{H}$  NMR data for compounds **1-3** (at 600 MHz in  $\text{CDCl}_3$ ,  $\delta$  in ppm, *J* in Hz).

| Position (H) | <b>1</b>                                  | <b>2</b>                             | <b>3</b>                             |
|--------------|---|--------------------------------------|--------------------------------------|
| 3a           | 2.10 (3H, <i>s</i> )                      | 2.12 (3H, <i>s</i> )                 | 2.09 (3H, <i>s</i> )                 |
| 4            | 7.04 (1H, <i>s</i> )                      | 7.33 (1H, <i>s</i> )                 | 7.20 (1H, <i>s</i> )                 |
| 5a           | 2.06 (3H, <i>s</i> )                      | 4.46 (2H, <i>s</i> )                 | 4.42 (2H, <i>s</i> )                 |
| 1'           |   |                                      | 2.89(1H, <i>m</i> )                  |
| 1'a          | 1.97 (3H, <i>s</i> )                      | 1.93 (3H, <i>s</i> )                 | 1.24 (3H, <i>d</i> , <i>J</i> = 6.8) |
| 2'           | 5.69 (1H, <i>d</i> , <i>J</i> = 6.4)      | 5.71 (1H, <i>t</i> , <i>J</i> = 7.3) | 1.75 (1H, <i>m</i> )                 |
|              |   |                                      | 1.53 (1H, <i>m</i> )                 |
| 3'           | 4.74 (1H, <i>quint.</i> , <i>J</i> = 6.4) | 2.22 (2H, <i>m</i> )                 | 1.29 (1H, <i>m</i> )                 |
|              |   |                                      | 1.22 (1H, <i>m</i> )                 |
| 4'           | 1.34 (3H, <i>d</i> , <i>J</i> = 6.4)      | 1.06 (3H, <i>t</i> , <i>J</i> = 7.5) | 0.89(3H, <i>t</i> , <i>J</i> = 7.3)  |

### 3.2 Cytotoxicity and Antimicrobial activity

HeLa cells were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. The cells were maintained at 37°C in a humidified atmosphere at 95% air and 5%  $\text{CO}_2$ . Cell viability was measured by MTT assay[8].

Compound **1** showed no effect on the growth of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and yeasts (*Candida albicans* and *Aspergillus niger*) at the concentration of 50  $\mu\text{g}/\text{disc}$ . Compound **1** exhibited week cytotoxicity against HeLa cells (10  $\mu\text{g}/\text{mL}$ , 2.73% and 20  $\mu\text{g}/\text{mL}$ , 7.39%) in MTT assay.

**Table 2.**  $^{13}\text{C}$  NMR data for compounds **1-3** (at 150 MHz in  $\text{CDCl}_3$   $\delta$  in ppm).

| Position (C) | 1                       | 2                                    | 3                                    |
|--------------|-------------------------|--------------------------------------|--------------------------------------|
| 2            | 163.3 (C)               | 163.2 (C)                            | 163.8 (C)                            |
| 3            | 123.6 (C)               | 123.5 (C)                            | 122.8 (C)                            |
| 3a           | 16.3 (CH <sub>3</sub> ) | 16.5 <sup>a</sup> (CH <sub>3</sub> ) | 16.4 <sup>a</sup> (CH <sub>3</sub> ) |
| 4            | 144.5 (CH)              | 144.9 <sup>b</sup> (CH)              | 144.8 <sup>b</sup> (CH)              |
| 5            | 110.4 (C)               | 114.0 <sup>c</sup> (C)               | 113.9 <sup>c</sup> (C)               |
| 5a           | 16.6 (CH <sub>3</sub> ) | 60.3 (CH <sub>2</sub> )              | 59.7 (CH <sub>2</sub> )              |
| 6            | 157.3 (C)               | 161.1 (C)                            | 164.3 (C)                            |
| 1'           | 128.4 (C)               | 126.7 (C)                            | 34.4 (CH)                            |
| 1'a          | 14.9 (CH <sub>3</sub> ) | 14.5 (CH <sub>3</sub> )              | 18.9 (CH <sub>3</sub> )              |
| 2'           | 138.8 (CH)              | 138.7 (CH)                           | 36.7 (CH <sub>2</sub> )              |
| 3'           | 64.7 (CH)               | 21.7(CH <sub>2</sub> )               | 20.8(CH <sub>2</sub> )               |
| 4'           | 23.1 (CH <sub>3</sub> ) | 13.5 <sup>d</sup> (CH <sub>3</sub> ) | 13.9 <sup>d</sup> (CH <sub>3</sub> ) |

<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, and <sup>d</sup> Interchangeable.

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## Supporting Information

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

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