SHORT REPORT

A C G publications

Rec. Nat. Prod. 17:5 (2023) 952-957

records of natural products

Nocardiopyrone C, a New Antimicrobial Pyran-2-one Derivative from a Marine-derived Actinomycete Strain *Nocardiopsis aegyptia* ZSN1

Yuhong Tian [#1], Ziwen Chen [#1], Mingzhu Ma [2] and Yongjun Jiang [1*]

[1] *School of Food and Pharmacy, Zhejiang Ocean University, Zhoushan 316022, P. R. China*

[2] *Zhejiang Marine Development Research Institute, Zhoushan 316022, P. R. China*

(Received April 20, 2023; Revised June 29, 2023; Accepted July 02, 2023)

Abstract: A new pyran-2-one derivative, Nocardiopyrone C (**1**), and three known compounds, Nocardiopyrone B (**2**), 1-hydroxy-4-methoxy-2-naphthoic acid (**3**), and 2-(aminocarbonyl)-phenylcarbamate (**4**) were identified from the marine-derived *Nocardiopsis aegyptia* ZSN1. The chemical structure of the novel compound (**1**) was ascertained as (7*R*)-5-(methoxymethyl)-3-methyl-6-(pentan-2-yl)-2*H*-pyran-2-one using HRESIMS data, spectroscopic data, and ECD calculations. All four isolated compounds displayed antimicrobial activity, with the new compound **1** having the same MIC value of 50.0 μM against *E. coli*, *S. aureus*, and *C. albicans*.

Keywords: *Nocardiopsis aegyptia*; nocardiopyrone C; antimicrobial.

1. Plant Source

The strain ZSN1 (Figure S1 in the supplementary data) was obtained from the sediments found in Zhoushan Island of Zhejiang province utilizing the conventional dilution plating technique. TaKaRa Biotechnology (Dalian, China) Co., Ltd. identified the strain through 16S rDNA sequence analysis, and its 16S rDNA sequence (Figure S2) was re-verified using ezbiocloud (ezbiocloud.net). Additionally, related sequences were aligned and analyzed using MEGA (Version X). Based on the results (Figure S3), we confirmed that strain ZSN1 is part of the *Nocardiopsis aegyptia* genus. The ZSN1 strain's sequence has been registered in GenBank with the accession number MN736491.

2. Previous Studies

The genus *Nocardiopsis*, first described by J. Meyer in 1976, belongs to the class Actinobacteria, order Actinomycetales, and family Nocardiopsaceae [1]. This genus is known for producing a diverse range of secondary metabolites with various biological activities. These secondary metabolites primarily consist of polyketides, diketopiperazines, pyrones, alkaloids, naphthoquinones, and phenoxazine derivatives. They exhibit a broad spectrum of pharmacological and biological effects, such as antibacterial, antifungal, and anticancer properties, and some play a crucial role in advancing pharmaceutical research [2-4].

* Corresponding author: E-Mail: jiangyj@zjou.edu.cn
# These authors contributed equally to this work

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products September-October 2023 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.405.2304.2763
Available online: July 11, 2023.

Throughout our continuous investigation for uncovering novel bioactive natural substances from oceanic microorganisms, we isolated strain ZSN1 from a marine sediment sample [5-11]. We prepared an organic extract from this strain's culture in Gauze's liquid medium, which exhibited antimicrobial properties. After additional chemical scrutiny of the bioactive extract, we identified four compounds, including a new bioactive pyran-2-one derivative. In this study, we present the isolation and cultivation of strain ZSN1, along with the extraction, structural examination, and antimicrobial properties of the obtained compounds.

3. Present Study

In this experiment, a single colony of the ZSN1 strain was cultured on a Gause's agar plate. Then it was inoculated into 250 mL of Gause's liquid medium in a 500 mL Erlenmeyer flask. The flask was then placed in an incubator at 28°C for a period of 10 days, during which fermentation took place on a rotary shaker operating at 180 rpm. After fermentation, the 20 L culture was divided into mycelia and supernatant through centrifugation. Both parts were extracted with methanol and ethyl acetate to obtain an organic extract (6.8 g). This extract was applied to a silica gel column (500 mm × 60 mm) and eluted with dichloromethane-methanol (100:1, 50:1, 30:1, 20:1, 10:1, 5:1, and 1:1, each 250 mL) to yield five fractions A−E. Fraction C (182.3 mg) underwent purification using preparative HPLC with a Pursuit 100Å C18 column (50.0 × 250 mm, 10 μm; mobile phase 15−100% methanol in water, 40 min; flow rate 10 mL/min; detection wavelength 230 nm), resulting in compounds **1** (6.2 mg, t_R= 30.5 min) and **2** (8.5 mg, t_R= 25.4 min), respectively. Fraction D, weighing 103.2 mg, was purified using the same column in preparative HPLC, but with a different mobile phase consisting of 47% methanol in water and a detection wavelength of 280 nm, obtaining compound **3** (6.6 mg, t_R = 26.0 min). Lastly, compound **4** (6.7 mg, t_R = 15.5 min) was obtained from Fraction E, which weighed 580.0 mg, using preparative HPLC with a mobile phase consisting of 10%-100% methanol in water and a detection wavelength of 254 nm.

Nocardiopyrone C ***(1)****:* Yellow oil; $[\alpha]_D^{20}$ = – 77.6 (*c* = 0.5, Methanol); UV (MeOH): 222 (3.8), 301 (4.1). ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS: *m/z* 225.1487 $[M+H]^+$ (calcd for *m/z* 225.1491) and *m/z* 247.1306 $[M+Na]^+$ (calcd for *m/z* 247.1310).

An organic extract, sourced from the cultures of ZSN1 in Gauze's liquid medium, was subjected to several rounds of separation using silica gel and Sephadex LH-20 column chromatography, and then purified through HPLC. This process led to the isolation of compounds **1-4**, as shown in Figure 1.

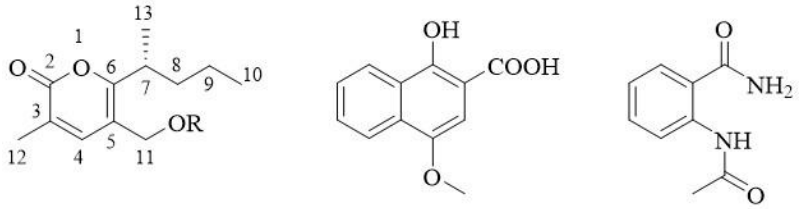

Figure 1. The structure of compounds **1-4**

Compound **1** was obtained as a yellow oil, with a measured $[\alpha]^{20}_D$ of **1** was -77.6° (*c* = 0.5, Methanol). The HRESIMS data for **1** had $[M+H]^+$ and $[M+Na]^+$ ion peaks at *m/z* 225.1487 (calcd. *m/z* 225.1491) and 247.1306 (calcd. *m/z* 247.1310), respectively. These data suggest that the compound **1** has a molecular formula of $C_{13}H_{20}O_3$, which is consistent with four degrees of unsaturation. The ^{1}H-NMR data (Table 1 and Figures S4-6) of compound **1** showed typical signals for 1-methylbutyl group [δ_H 0.90 (3H, t, *J* = 7.2 Hz, Me-10), 1.20 (3H, d, *J* = 6.6 Hz, Me-13), 1.30 (1H, m, H-9), 1.21 (1H, m, H-9), 2.97 (1H, ddq, *J* = 10.1, 8.8, 6.6 Hz, H-7), 1.68 (1H, ddt, *J* = 13.2, 9.0, 5.6 Hz, H-8), 1.51 (1H, ddt, *J* = 13.3, 10.0, 5.8 Hz, H-8)], a methoxy located at δ_H 3.34 (3H, s), one aromatic methyl proton (δ_H 2.03, s, Me-12), one oxygenated methylene (δ_H 4.22, 4.19, 2dd, *J* = 12.0, 2.1 Hz, H-11), and one aromatic proton (δ_H 7.32, s, H-4). The ^{13}C

NMR combined with HSQC spectra showed 13 carbon atoms (Table 1 and Figures S7-S8), consisted of four methyl, three methylenes, two methines, and four quaternary carbons. Given the characteristic signals for pyran-2-one nucleus at δ_C 165.9 (s, C-2), 123.2 (s, C-3), 144.9 (d, C-4), 114.1 (s, C-5) and 166.3 (s, C-6), compound **1** should be a pyran-2-one derivative [12-14].

The structure of compound **1** was ascertained through the examination of 2D NMR data (Figure 2). The 1-methylbutyl group was linked to C-6, as suggested by the HMBC correlations of H-8, H-5, and Me-13 with C-6, while the methoxymethyl was connected to C-5, as indicated by the HMBC cross-peaks of H-11 with C-5, C-6, and C-7, and Me-14 with C-11 (Figure 2 and Figure S9). The pyran ring was confirmed by HMBC cross-peaks of H-4 with C-2 and C-6 (Figure 2 and Figure S9). Finally, Me-12 was linked to C-3, as suggested by the HMBC cross-peaks of Me-12 with C-4, C-3, and C-2 (Figure 2 and Figure S9). The ^{1}H-^{1}H COSY spectrum displayed two spin system, namely, H-7/Me-13 and H-7/H-8/H-9/Me-10 (Figure 2 and Figure S10). As a result, the planar structure of **1** was established as 5-(methoxymethyl)-3-methyl-6-(pentan-2-yl)-2*H*-pyran-2-one.

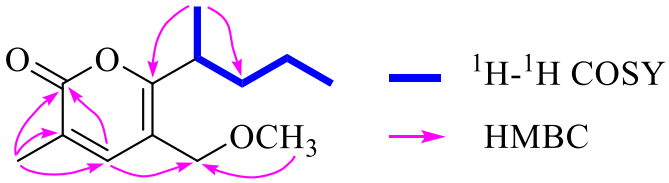

Figure 2. Essential 2D NMR correlations for **1**

The C-7 absolute configuration of compound **1** was established through ECD calculations. The theoretical ECD spectra calculations for compound **1** were performed using Gaussian and visualized using GaussView. A conformational search yielded six low-energy conformers of 7*R*-**1**, which were then optimized through density functional theory (DFT) at the B3LYP/6-31+g (d, p) level in MeOH environment. In the same MeOH environment, we performed theoretical ECD computations using Time-dependent Density Functional Theory (TD-DFT) at the B3LYP/6-311+g (d, p) level for all the conformations of **1**. As depicted in Figure 3, the calculated ECD curve of 7*R*-**1** closely match with experimental curve of **1**. Besides, both **1** and co-isolated Nocardiopyrone B (**2**) had similar experimental ECD curves (Figure 3). Thus, the structure of **1** was elucidated as (7*R*)-5-(methoxymethyl)-3-methyl-6-(pentan-2-yl)-2*H*-pyran-2-one, named as Nocardiopyrone C, a new member of pyran-2-one family.

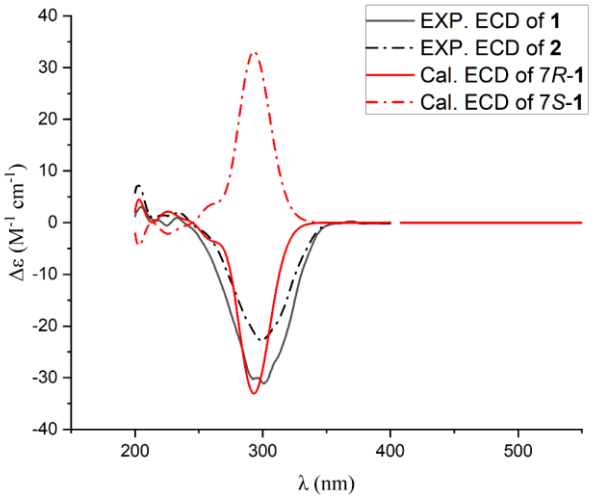

Figure 3. Experimental ECD spectra of compounds **1** and **2** (200-500 nm) along with calculated ECD spectra of compound **1**

Table 1. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR data of compounds **1** and **2** were obtained in Methanol-d_4, and the comparison NMR data for compound **2** were collected in DMSO-d_6 from the literature [12].

	1		**2**			**2** [12]	
position	**δ_H, *J* in Hz**	**δ_C, type**	**δ_H, *J* in Hz**	***δ* (C)**	**δ_H, *J* in Hz**	**δ_C, type**	
2			165.9 (s)			165.2 (s)	163.0 (s)
3			123.2 (s)			123.1 (s)	121.6 (s)
4	7.32 (s)		144.9 (d)	7.37 (s)		144.7 (d)	7.34 (s)	143.5 (d)
5			114.2 (s)			116.8 (s)	115.7 (s)
6			166.3 (s)			166.0 (s)	163.4 (s)
7	2.97 (ddq, *J* = 10.1, 8.8, 6.6)		35.4 (d)	2.99 (ddq, *J* = 10.1, 8.8, 6.6)		35.3 (d)	2.93 (ddq, *J* = 10.1, 8.8, 6.6)	33.7 (d)
8	1.51 (ddt, *J* = 13.3, 10.0, 5.8) 1.68 (ddt, *J* = 13.2, 9.0, 5.6)		38.0 (t)	1.50 (ddt, *J* = 13.3, 10.0, 5.8) 1.67 (ddt, *J* = 13.2, 9.0, 5.6)		38.0 (t)	1.41 (ddt, *J* = 13.3, 10.0, 5.8) 1.54 (ddt, *J* = 13.2, 9.0, 5.6)	36.9 (t)
9	1.30, 1.21 (2m)		21.8 (t)	1.23, 1.30 (2m)		21.7 (t)	1.14, 1.22 (2m)	20.6 (t)
10	0.90 (t, *J* = 7.2)		14.4 (q)	0.90 (t, *J* = 7.3)		14.3 (q)	0.83 (t, *J* = 7.3)	14.4 (q)
11	4.22, 4.19 (2dd, *J* = 12.0, 2.1)		69.8 (t)	4.38, 4.32 (2dd, *J* = 12.4, 5.0)		59.4 (t)	4.23, 4.18 (2dd, *J* = 12.4, 5.0)	58.0 (t)
12	2.03 (s)		16.2 (q)	2.04 (s)		16.2 (q)	1.95 (s)	16.5 (q)
13	1.20, (d, *J* = 6.6)		19.3 (q)	1.12 (d, *J* = 6.6)		19.2 (q)	1.11 (d, *J* = 6.6)	19.2 (q)
11-OH							5.10 (t, *J* = 5.0)	
11-OCH_3	3.34 (s)		58.3 (q)					

As solvent methanol was used in the process of extraction, it was possible that Nocardiopyrone C (**1**) might be an artifact derived from Nocardiopyrone B (**2**) [12]. To explore this possibility, an organic ethanol extract from the ZSN1 strain was examined using HPLC-HRESIMS. The data indicated that Nocardiopyrone C (**1**) was present in the ethanol crude extract (Figure S13), confirming that **1** was not an artifact but a new natural product.

Through NMR spectroscopic analyses, HRESIMS, and comparison with published papers, compounds **2-4** were identified as as Nocardiopyrone B (**2**) [12], 1-hydroxy-4-methoxy-2-naphthoic acid (**3**) [15] and 2-(aminocarbonyl)-phenylcarbamate (**4**) [16], respectively.

Compounds **1-4** were assessed for their activity against the growth of *E. coli* (ATCC 25922), *S. aureus* (ATCC 43300), and *C. albicans* (ATCC 10231) by employing inhibition zones and micro-broth dilution methods, as described before [9, 17,18]. Amphotericin B (a polyene antifungal drug) and chloramphenicol (an organochlorine antibacterial drug) were utilized as positive controls. The findings (Table 2) revealed that the novel compound **1** displayed antimicrobial activities with identical MIC values of 50.0 μM against *E. coli*, *S. aureus*, and *C. albicans*. Nocardiopyrone B (**2**), 1-hydroxy-4-methoxy-2-naphthoic acid (**3**), and 2-(aminocarbonyl)-phenylcarbamate (**4**) also exhibited antibacterial effects on *E. coli* and *S. aureus*, as well as antifungal effects on *C. albicans*, as depicted in Table 2.

Table 2. Antimicrobial activities of compounds **1-4** and the antimicrobial data for compound **2** obtained from the literature [12].

Compound	MIC (μM)		
	Escherichia coli	***Staphylococcus aureus***	***Candida albicans***
1	50.0	50.0	50.0
2	50.0	50.0	25.0
2 *	48.0	48.0	24.0
3	50.0	50.0	>100
4	50.0	>100	>100
Ampicillin	0.88	7.2	-
Amphotericin B	-	-	13.5

Data from the literature [12]

Acknowledgments

This work was partially supported by Basic Public Welfare Research Project of Zhejiang Province (No. LGF22H300005), National Undergraduate Training Program for Innovation and Entrepreneurship (No. 202110340033), Zhejiang Provincial Leading Innovation and Entrepreneurship Team Project (No. 2020R01015), the Key Research and development Program of "Leading Goose" of Zhejiang Province (No. 2022C02059), and the project of Bureau of Science and Technology of Zhoushan (No. 2021C41008).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Yuhong Tian: 0009-0000-8101-4727
Ziwen Chen: 0009-0001-0475-1923
Mingzhu Ma:0009-0004-6038-7435
Yongjun Jiang: 0000-0001-5419-7345

References

[1] T. Bennur, A. R. Kumar, S. Zinjarde and V. Javdekar (2015). Nocardiopsis species: Incidence, ecological roles and adaptations. *Microbiol. Res.* **174**, 33-47.
[2] T. Bennur, R. Kumar, Z, S. S. Zinjarde and V. Javdekar (2016). Nocardiopsis species: a potential source of bioactive compounds. *J. Appl. Microbiol.* **120**, 1-16.
[3] R. Raju, A. Piggott, X. Huang, R. Capon (2011). Nocardioazines: a novel bridged diketopiperazine scaffold from a marine-derived bacterium inhibits P-glycoprotein. *Org. Lett.* **13**, 2770-2773.
[4] R. Subramani, D. Sipkema (2019). Marine rare actinomycetes: A promising source of structurally diverse and unique novel natural products. *Mar. Drugs.* **17**, 249.
[5] Y. Tian, Y. Jiang, Z. Wen, L. Guan, X. Ouyang, W. Ding, Z. Ma (2023). Identification of novel sphydrofuran-derived derivatives with lipid-lowering activity from the active crude extracts of *Nocardiopsis* sp. ZHD001. *Int. J. Mol. Sci.* **24**, 2822.
[6] X. Cheng, J. Li, Y. Jiang, H. Liu, C. Huo (2021). A new indolizinium alkaloid from marine-derived *Streptomyces* sp. HNA39. *J. Asian Nat. Prod. Res.* **23**, 913-918.
[7] Y. Jiang, Y. Huang, S. Chen, Y. Ji, Z. Ma (2020). Strepolyketides A-C, three novel SEK15-derived polyketides from *Streptomyces* sp. HN2A53. *Tetrahedron Lett.* **61**, 151996.
[8] Y. Jiang, Y. Ji, J. Li, W. Ding (2020). A new compound from the marine-derived *Streptomyces* sp. SS13M, *J. Asian Nat. Prod. Res.* **22**, 701-706.
[9] Y. Jiang, D. Zhang, H. Zhang, J. Li, W. Ding, C. Xu, Z. Ma (2018). Medermycin-type naphthoquinones from the marine-derived *Streptomyces* sp. XMA39. *J. Nat. Prod.* **81**, 2120-2124.
[10] Y. Jiang, J. Li, H. Zhang, W. Ding, Z. Ma (2018). Cyclizidine-type alkaloids from *Streptomyces* sp. HNA39. *J. Nat. Prod.* **81**, 394-399.
[11] Y. Jiang, L. Gan, W. Ding, Z. Chen, Z. Ma (2017). Cytotoxic gephyromycins from the *Streptomyces* sp. SS13I. *Tetrahedron Lett.*, **58**,3747-3750.
[12] Z. Wang, P. Fu, P. Liu, P. Wang, J. Hou, W. Li, W. Zhu (2013). New pyran-2-ones from alkalophilic actinomycete, *Nocardiopsis alkaliphila* sp. Nov. YIM-80379. *Chem. Biodivers.* **10**, 281-287.
[13] C. Lin, C.H. Lu and Y.M.Shen, YM (2010). Three new 2-pyranone derivatives from mangrove endophytic Actinomycete strain Nocardiopsis sp A00203, *Rec. Nat. Prod*. **4**, 176-179.
[14] H.S. Chung, L.T. Hsiao, Z.Y. Li and Z.H. Wen (2021). A new angular naphthopyrone from crinoid Colobometra perspinosa, *Rec. Nat. Prod*. **15**, 213-218.
[15] C. Pfefferle, J. Breinholt, H. Gurtler, H. Fiedler (1997). 1-Hydroxy-4-methoxy-2-naphthoic acid, a herbicidal compound produced by *Streptosporangium cinnabarinum* ATCC 31213. *J. Antibiot.* **50**, 1067-1068.
[16] N. Phay, H. Yada, T. Higashiyama, A. Yokota, A. Ichihara, F. Tomita (1996). NP-101A, antifungal antibiotic from *Streptomyces aurantiogriseus* NPO-101. *J. Antibiot.* **49**, 703-705.
[17] Y.P. Li, S.L. Sheng, J. Feng, Y.D. Wang, J. Guo, Y.T. Jiang and W.G. Wang (2022). New cyclic peptides from the endophytic *Aspergillus versicolor* 0312 with their antimicrobial activity, *Rec. Nat. Prod*. **16**, 585-591.
[18] C. Liu, J. Ling, L. Yang, X. Ouyang, N. Wang (2023). Chitosan-based carbon nitride-polydopamine-silver composite dressing with antibacterial properties for wound healing. *Carbohyd. Polym.* **303**, 120436.