SHORT REPORT

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Streptolactone A, A New Antibiofilm Lactone Derivative from *Streptomyces* sp. A31

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Abstract: A novel lactone derivative, named Streptolactone A (**1**), was unearthed from *Streptomyces* sp. A31 isolated from marine sediments. Its structure was elucidated through a comprehensive analysis of 1D, 2D NMR, and HRESIMS data. Assessment of its antibiofilm and antibacterial effects against *P. aeruginosa* was conducted via the microdilution and crystal violet staining method, revealing Streptolactone A's notable potency in inhibiting biofilm formation.

Keywords: *Streptomyces* sp.; Streptolactone A; antimicrobial; antibiofilm.

1. Bacteria Material

The strain A31 was isolated from sediment samples collected near Zhoushan, China. Using the gradient dilution plate method, the strain was purified and then cultured on sterile Gauze's No. 1 agar plates at 30°C for 3-5 days. The strain grew as white mycelia on the medium. Strain identification was performed through morphological observation and 16S rDNA sequencing, which was outsourced to BGI Co., Ltd. The obtained sequence was compared with known sequences in the GenBank database, revealing a 100% similarity to *Streptomyces bacillaris*. Consequently, the strain was identified as *Streptomyces* sp. The sequence has been submitted to the GenBank database with the accession number PP077262. A voucher strain, designated *Streptomyces* sp. A31, is meticulously maintained at the Laboratory of the School of Medicine, Yangzhou Polytechnic College, Yangzhou, China.

2. Previous Studies

Pseudomonas aeruginosa is a highly adaptive and opportunistic pathogen renowned for its ability to form biofilms on medical devices and human tissues [1]. The formation of biofilms significantly enhances the bacterial community's resistance to antibiotics and host immune responses, thereby rendering infections difficult to treat [2]. The ability to form biofilms is a key virulence factor for *Pseudomonas aeruginosa*, as biofilms shield the bacteria from environmental stressors, including antibiotic treatments [3]. Hence, developing and applying anti-biofilm agents have become highly effective strategies for treating drug-resistant bacterial infections.

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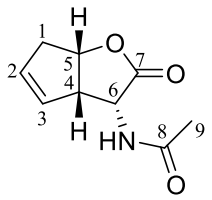
To harness bioactive compounds from marine-derived microorganisms[4-7], A strain identified as Streptomyces sp. A31 was isolated from a marine sediment sample collected on Zhoushan Island. The ethyl acetate (EtOAc) extract, derived from the rice solid media culture of this strain, demonstrated notable antimicrobial activities, along with a significant impact on the biofilm-forming capabilities of *Pseudomonas aeruginosa*. This led to the discovery of a novel natural product endowed with antibiofilm properties. This study details the isolation process, structural elucidation, and the biological attributes of this newly identified compound.

3. Present Study

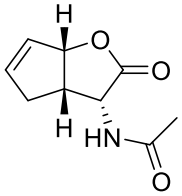
In this experiment, *Streptomyces* sp. A31 was cultivated on Gauze's agar plates, which were prepared by dissolving soluble starch (20.0g), KNO_3 (1.0g), KH_2PO_4 (0.5g), $MgSO_4$ (0.5g), NaCl (0.5g), $FeSO_4{\cdot}7H_2O$ (0.01g), and sea salt (25.0g), in distilled water to make up 1 L, solidified with agar (20.0g). After inoculation, the strain was fermented on a sterile rice solid medium in 500 mL Erlenmeyer flasks, each containing 40 g of rice and 60 mL of 2.5% sea salt solution, for approximately two months at 28°C across a total of 100 flasks. The cultured media were extracted thrice with ethyl acetate, after which the residual organic solvent was removed through evaporation. The resulting extracts (10.6 g) were then subject to fractionation using a silica gel column chromatography, employing a gradient of CH_2Cl_2 and MeOH (100:1 to 0:1 ratios, with 500 mL for every step), yielding thirteen combined fractions (A-M). Fraction E was further purified using preparative HPLC under conditions of MeOH/H_2O+0.05% TFA, gradient from 10% to 90% over 40 minutes at a flow rate of 10 mL/min, to yield compound **1** (1.9 mg, retention time = 19.1min).

*Streptolactone A (**1**)*: Yellowish oil. $[\alpha]_D^{20}$ = – 69.3 (*c* = 0.12, Methanol) .^{1}H NMR and ^{13}C NMR data, Table 1; HRESIMS data: *m/z* 182.0807 $[M+H]^+$ (calcd for *m/z* 182.0812) and *m/z* 385.1346 $[2M+Na]^+$ (calcd for *m/z* 385.1370).

Crude extract from rice solid media was separated and purified by silica gel column and preparative HPLC to give single compound, eventually one new compound **1** was obtained. Its chemical structure was given in Figure 1.



Streptolactone A



2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-endo-carboxylate[8]

Figure 1. Chemical structure of streptolactone (**1**) and its known derivative

Compound **1** was obtained as yellowish oil. The molecular formula was $C_9H_{11}NO_3$ deduced from its HRESIMS data at *m/z* 182.0807 $[M+H]^+$ (calcd for 182.0812), representing five degrees of unsaturation. The IR spectrum showed absorption bands indicative of functionalities associated with a double bond (1589 cm^{-1}) and amino reactive hydrogen (3460 cm^{-1}). The ^{1}H NMR data (Table 1) in $CDCl_3$ indicated one NH protons [δ_H 6.21], one methyl [δ_H 2.06 (s, Me-9)], two olefin protons [δ_H 5.82 (m, H-3); δ_H 5.77 (m, H-2)], two nonequivalent methylene protons [δ_H 2.77 (m, H-1a); δ_H 2.67 (dt, *J* = 18.5,1.7, H-1b)] and three methylenes [δ_H3.87 (dd, *J* = 6.4, 3.5 Hz, H-6); δ_H 3.44 (m, H-4); δ_H 5.38 (td, *J* =6.8, 1.7, H-5)]. The ^{13}C NMR data combine with HSQC correlations showed nine carbon atoms, including two carbonyl carbons (δc 175.1, C-7; δc170.4, C-8), a couple of olefin carbons (δc 130.8, C-

3; δc 130.1, C-2) and other five carbons (δc 54.8, C-6; δc 53.6, C-4; δc 39.1, C-1; δc82.7, C-5; δc 22.7, C-9).

Through comprehensive analysis of HSQC, COSY, and HMBC correlations, the planar structure of compound **1** was determined (Figure 2). The COSY spectrum revealed a unique spin system comprising H-1, H-2, H-3, H-4, and H-5, indicating the presence of a cyclopentene moiety. HMBC correlations from H-6 to C-3 and C-7, along with a crucial COSY correlation between H-4 and H-6, suggested the incorporation of a lactone ring fused with the cyclopentene rings at C-4 and C-5. Additionally, HMBC correlations from H-9 to C-8 and between H-6 and C-8 revealed an acetyl amino group attached to C-6. The observation of a splitting peak for the active amino hydrogen atom (δ_H 6.21) on H-3 (δ_H 3.87, dd, J = 6.4, 3.5 Hz) in $CDCl_3$ further supported this structure. Hence, the planar structure of compound 1 was elucidated as depicted.

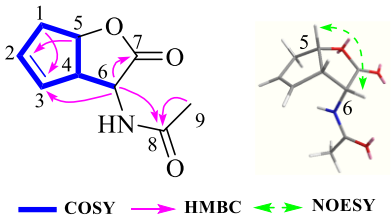


Figure 2. Key 2D correlations of compound **1**

Through meticulous evaluation of vicinal coupling constants and NOESY correlations, we established the relative configuration of C-4, C-5, and C-6 for compound **1**. The significant coupling constant between H-4 and H-6 ($J_{H4\text{-}H6}$ = 1.7 Hz) suggested a *cis* conformation, while the observed NOESY correlation between H-5 and H-6 indicated that these hydrogens are on the same side of the molecule. Consequently, the structure of compound **1** was conclusively identified and has been named Streptolactone A.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1**, and the comparison NMR data for 2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-*endo*-carboxylate were collected in $CDCl_3$ from the literature[8]

Position	**1[a]**		**1[b]**		**2-acetyl-2-azabicyclo[2.2.1]hept-5-ene-3-endo-carboxylate[8]**	
	δ_H (J in Hz)	**δ_C, type**	**δ_H (J in Hz)**	**δ_C, type**	**δ_H (J in Hz)**	**δ_C, type**
1a	2.77, m	39.1, CH_2	2.77, m	40.3, CH_2	6.22, t, 2.5	140.5, CH
1b	2.67, dt (18.5, 1.7)		2.61, d (18.3)			
2	5.77, m	130.1, CH	5.73, m	131.6, CH	5.90-6.0, m	127.7
3	5.82, m	130.8, CH	5.83, m	131.8, CH	2.47, m	32.0, CH_2
					2.31, m	
4	3.44, m	53.6, CH	3.36, m	55.4, CH	3.52, m	40.5, CH
5	5.38, td (6.8, 1.7)	82.7, CH	5.34, t (6.3)	84.8, CH	5.40, t (2.0)	87.3, CH
6	3.87, dd (6.4, 3.5)	54.8, CH	3.91, d (2.8)	55.7, CH	4.78, d (4.8)	52.8
7		175.1, C		177.5, C		170.4
8		170.4, C		173.5, C		no report
9	2.06, s	22.7, CH_3	1.99, s	22.4, CH_3	2.08, s	22.7, CH_3
6-NH	6.21, d, J = 6.4				6.11, br s	

[a, b] Measured in $CDCl_3$ and CD_3OD respectively.

Compound **1** was tested for its antibiofilm and antibacterial efficacy against *P. aeruginosa* PA01. The results revealed that compound 1 exhibited potent antibiofilm activity, achieving a minimum effective concentration of 87.5 μM and inhibiting biofilm formation by 22.3% (Figure 3). Notably, at a

concentration of 1 mM, compound 1 demonstrated no significant antibacterial activity. In contrast, the positive control, ciprofloxacin, exhibited a clear inhibitory effect with a MIC value of 3.5 μM.

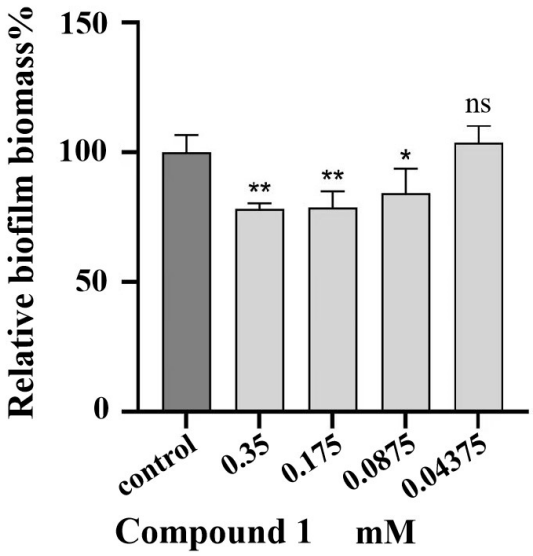


Figure 3. The effects on biofilm formation under various concentrations of compound **1** were evaluated, and the results were compared with those of the control group (DMSO) *p < 0.05;***p < 0.005;****p < 0.0001; ns denotes no significant difference (p > 0.05)

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Supporting Information

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

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