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Actinolactomycin, a New 2-Oxonanonoidal Antitumor Antibiotic Produced by *Streptomyces flavoretus* 18522, and its Inhibitory Effect on the Proliferation of Human Cancer Cells

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Abstract: Actinolactomycin **1**, a new 2-oxonanonoidal antitumor antibiotic, was isolated from the fermentation broth of *Streptomyces flavoretus* 18522 through a bioassay-guided separation procedure. The structure of **1** was determined as 4,7-dihydroxy-3,9-dimethyl-2-oxonanone by the spectroscopic methods. Compound **1** inhibited the proliferation of A2780, K562, HCT-15, A549 and HeLa cells with the IC_{50} values of 1.4 ± 0.4 μmol/L, 8.4 ± 4.7 μmol/L, 9.4 ± 2.2 μmol/L, 15.4 ± 5.6 μmol/L and 13.7 ± 2.0 μmol/L, respectively. Flow cytometric analysis indicated that **1** could inhibit the cell cycle of tsFT210, A2780 and K562 cells mainly at the G0/G1 phase and could also induce apoptosis in K562 cells.

Keywords: Actinolactomycin, 2-oxonanonoid, structure, antitumor agent, cell cycle inhibitor, apoptosis inducer, *Streptomyces flavoretus*.

In the course of our screening for new anticancer agents from microbial resources using mammalian cancer tsFT210 cells[1-3], we found that the fermentation broth of an actinomycete strain 18522 significantly inhibited the cell cycle of tsFT210 cells at the G0/G1 phase. From the fermentation broth of this strain, we have now isolated a new 2-oxonanonoidal anitumor antibiotic, named actinolactomycin **1**, through a bioassay-guided separation procedure. In this communication, the isolation, structure determination and brief biological property of **1** were described.

The producing strain 18522, isolated from a soil sample collected in Yunnan Province, China, was identified as *Streptomyces flavoretus* through a taxonomic study and has been deposited at the China General Microbiological Culture Collection Center with the registration number of CGMCC No.1022.

The producing strain, *Streptomyces flavoretus*18522 CGMCC 1022, was inoculated into two 500 mL Erlenmeyer flasks containing 100 mL of seed medium consisting of 0.5% soybean meal, 1.5% soluble starch, 1.5% glycerol, 1.5% meat extract and 0.2% $CaCO_3$ (pH 7.4) and cultured on a rotary shaker at 28°C under 180 rpm for 2 days to obtain seed culture. The seed culture was seeded into one hundred 500 mL Erlenmeyer

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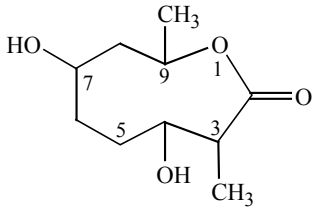
flasks, each containing 150 mL of fermentation medium with the same composition of the seed medium and they were fermented on a rotary shaker at 28°C under 180 rpm for 5 days to obtain fermentation broth (15 L). As the fermentation broth significantly inhibited the cell cycle of tsFT210 cells at the G0/G1 phase in a preliminary screening test, the following separation procedure was monitored by the same activity detected in the preliminary examination.

The whole broth (15L) was centrifuged to give broth supernate (13L) and a mycelial cake. The former supernate was extracted with the same volume of chloroform to obtain chloroform solution. The latter mycelial cake was extracted with 80% aqueous acetone at room temperature. The aqueous acetone solution obtained was evaporated *in vacuo* to remove acetone and then extracted with the same volume of chloroform to obtain chloroform solution. Both the chloroform solutions obtained were combined and concentrated *in vacuo* to afford an active chloroform extract (7.5 g). The whole chloroform extract was separated by vacuum liquid chromatography over silica gel H [bp 60-90°C petroleum ether-EtOAc (100:0→0:100)] and preparative HPLC on a SENSHU PAK ODS column (20 x 250 mm) using MeOH-H_2O (85:15 v/v) as eluting solvent at flow rate 10 mL/min under detector wavelength 215 nm to give a crude material. Further purification of the crude material by Sephadex LH-20 column chromatography with $CHCl_3$-MeOH (1:1 v/v), followed by recrystallization from acetone, gives pure **1** (30 mg).

Actinolactomycin (**1**), colorless needles, mp 152-153°C, $[\alpha]_D^{27}$ +1.22 (*c* 1.0, $CHCl_3$), gave *quasi*-molecular ion peaks at *m/z* 185 $[M+H-H_2O]^+$ and 203 $[M+H]^+$ in the positive TOF-MS and its molecular formula, $C_{10}H_{18}O_4$, could be determined by HR-TOF-MS measurement (measured 185.1182 $[M-H_2O+H]^+$, calcd. for $C_{10}H_{17}O_3$ ($[M+H-H_2O]^+$) 185.1178; measured 203.1261, calcd. for $C_{10}H_{19}O_4$ $[M+H]^+$ 203.1283). It showed the end absorption at 204 nm (log ε 3.17) in the UV spectrum in MeOH, and its IR spectrum (KBr) suggested the presence of esterified carboxylic carbonyl (1728 cm^{-1}) and hydroxyl groups (3380 cm^{-1}) in **1**.

The ^1H and ^{13}C NMR spectra of **1** in $CDCl_3$, coupled with the result of DEPT experiments (**Table 1**), indicated the presence of two methyl groups, three oxygenated methine, three methylene and a methine groups as well as an esterified carboxyl group. Analysis of the PFG ^1H-^1H COSY and PFG HMQC spectra (**Table 1**) enabled us to deduce partial structures A, a structural part in **1** related to the proton spin network, and B (**Figure 2**). Then, the connectivity of the partial structures could be determined by

Figure 1 The structure of actinolactomycin (**1**)



1

HMBC correlations (**Table 1**). In the PFG HMBC spectrum, the carbonyl carbon (C-2) in the partial structure B correlated with the protons, H-3, CH_3-3, H-4 and H-9, in the partial structure A, evidencing the carbon-carbon linkage between C-2 and C-3 and the ester linkage between C-2 and C-9 to form a lactonic ring in **1**. Taking account of the molecular composition given above, the structure of **1** could be determined as 4, 7-dihydroxy-3, 9-dimethyl-2-oxonanone as shown in **Figure 1**.

Figure 2 Partial structures for **1**

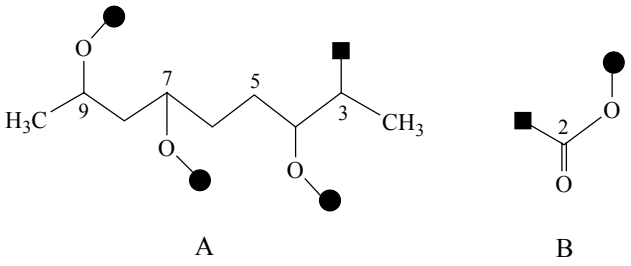


Table 1 600 MHz ^{1}H and 150 MHz ^{13}C NMR data for **1** in chloroform-*d* [a)]

Positions	δ_H (J in Hz)	^{1}H-^{1}H COSY[b)]	δ_C	HMBC[c)]	
				$^2J_{CH}$	$^3J_{CH}$
2	———		174.18		
3	2.50 m	$\underline{H}_3$C-3/H-4	45.19 d	H-3/C-2,4,$\underline{C}H_3$-3	H-3/C-5
4	4.00 q (7.3)	H-3/H-5	79.98 d	H-4/C-3	H-4/$\underline{C}H_3$-3,C-2
5	1.61 m	H-4/H-5(δ1.92)/H-6	28.10 t	H-5/C-4,C-6	H-5/C-3,C-7
	1.92 m	H-4/H-5(δ1.61)/H-6		H-5/C-4,C-6	H-5/C-3,C-7
6	1.48 m	H-5/H-6(δ1.99)/H-7	31.34 t	H-6/C-5,C-7	H-6/C-4,C-8
	1.99 m	H-5/H-6(δ1.48)/H-7		H-6/C-5	H-6/C-4
7	3.84 m	H-6/H-8	76.30 d		H-7/C-9
8	1.74 m	H-7/H-8(δ1.76)/H-9	42.22 t	H-8/C-7,C-9	H-8/C-6,$\underline{C}H_3$-9
	1.76 m	H-7/H-8(δ1.74)/H-9		H-8/C-7,C-9	H-8/C-6,$\underline{C}H_3$-9
9	4.96 m	H-8/$\underline{H}_3$C-9	69.00 d	H-9/C-8,$\underline{C}H_3$-9	H-9/C-2,C-7
3-CH_3	1.08 d (6.96)	H-3	12.80 q	$\underline{H}_3$C-3/C-3	$\underline{H}_3$C-3/C-2,C-4
9-CH_3	1.22 d (6.24)	H-9	20.46 q	$\underline{H}_3$C-9/C-9	$\underline{H}_3$C-9/C-8

a) Signal assignments were based on the results of DEPT, PFG ^{1}H-^{1}H COSY, PFG HMQC and PFG HMBC spectroscopy. Multiplicities of the carbon signals were determined by the DEPT method. b) Numbers in the column indicate the protons that showed correlations with the proton on the line in the PFG ^{1}H-^{1}H COSY. c) Numbers in the column indicate the carbons that correlated with the proton on the line through two ($^2J_{CH}$) and three ($^3J_{CH}$) bonds, respectively, which were detected in the PFG HMBC spectrum measured with the long-range J_{CH} = 8 Hz.

Actinolactomycin **1** inhibited the proliferation of A2780, K562, HCT-15, A549 and HeLa cells with the IC_{50} values of 1.4 ± 0.4 μmol/L, 8.4 ± 4.7 μmol/L, 9.4 ± 2.2 μmol/L, 15.4 ± 5.6 μmol/L and 13.7 ± 2.0 μmol/L, respectively, in the cell proliferation assay (SRB method)[4]. Flow cytometric analysis indicated that **1** could inhibit the cell cycle of tsFT210, A2780, and K562 cells mainly at the G0/G1 phase with the MIC values of 15.1 nmol/L, 0.1 μmol/L and 1.0 μmol/L, respectively, and could also induce apoptosis in K562 cells with the MIC value of 0.01 μmol/L.

There are 27 artificially synthesized 2-oxonanonoids, which do not contain OH groups[5,6]. Their biological activities have not been reported. Actinolactomycin **1** is a new 2-oxonanonoidal compound possessing antitumor activity, which provides the first example of naturally occurring 2-oxonanonoids, the first example of hydroxylated 2-oxonanonoids and also the first example of antitumor 2-oxonanonoids showing cell cycle inhibitory and apoptosis inducing activities.

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