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NOTE

A new sarkomycin analog from *Streptomyces* sp. HS-HY-144

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Sarkomycin is an antitumor antibiotic isolated from the culture filtrate of *Streptomyces erythrochromogenes*.[1–3] Until 1960s, sarkomycin was used as a prescription drug to treat cancer in Japan. Although sarkomycin exhibits strong inhibitory effect on several cancer cell lines, the activity is not effective enough.[3–5] A great effort has been extended to isolate or synthesize the analogous compounds of sarkomycin (Figure 1) expecting for more intensive antitumor chemicals with lower toxicity.[6–10] During the course of searching for novel microorganism-derived antitumor secondary metabolites, we investigated the chemical constituents of a strain *Streptomyces* sp. HS-HY-144. As a result, a new sarkomycin analog (**1**, Figure 1) was isolated from the fermentation broth of the strain. In this paper, the details of isolation and structure characterization of compound **1** are described.

Strain *Streptomyces* sp. HS-HY-144 was isolated from a soil sample collected from a farmland located in Tengchong, Yunnan province, China. The strain was identified as the genus *Streptomyces* because its 16S rDNA sequence (accession no: KR232515) exhibited a high sequence similarity of 100% with that of *S. mirabilis* strain 3662 (accession no: EF371431.1).

The strain *Streptomyces* sp. HS-HY-144 was maintained on the medium containing glucose (Sinopharm Chemical Reagent Co, Ltd, Shanghai, China) 10 g, maltose (Sinopharm Chemical Reagent Co, Ltd) 3 g, yeast extract (Oxoid Ltd, Basingstoke, UK) 3 g, $K_2HPO_4{\cdot}3H_2O$ 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, NaCl 0.5 g, KNO_3 1 g and agar (Becton Dickinson and company, Franklin Lake, NJ, USA) 20 g in 1.0 l of tap water, pH 7.0. The seed medium consisted of glucose 4 g, maltodextrin (Shandong Xiwang Group Ltd, Binzhou, Shandong, China) 10 g, yeast extract 4 g and $CaCO_3$ 2 g in 1.0 l water at pH 7.2–7.4. All the media were sterilized at 121 °C for 20 min. Slant culture was incubated for 6–7 days at 28 °C. Fermentation was carried out in a 50-l fermentor (containing 30 l of production medium; Shanghai Guoqiang Bioengineering Equipment, Shanghai, China; THS-50 l), tank pressure control at 0.05 MPa. The producing medium was composed of glucose 1%, soluble amylum (Haiyan Liuhe Starch Chemical Co, Ltd, Haiyan, China) 4%, yeast extract 0.5%, soybean powder (Ningbo Beilun Jiangnan Grease Co, Ltd, Ningbo, China) 2.5%, peptone (Sincere, Shanghai Sincere Biotech Co, Ltd, Shanghai, China) 0.5%, $CaCO_3$ 0.2%, $MgSO_4{\cdot}7H_2O$ 0.8%, $FeSO_4{\cdot}7H_2O$ 0.6%, $ZnSO_4{\cdot}7H_2O$ 0.2%, $MnSO_4{\cdot}H_2O$ 0.2% and $CoCl_2{\cdot}6H_2O$ 0.05%, $Na_2MoO_4{\cdot}2H_2O$ 0.2% at pH 7.0 before sterilization. The fermentation was conducted at 28 °C for 7 days by stirring at 100 r min^{-1} with an aeration rate of 900 l of air per hour.

The final 30 l of broth from the 50-l fermentor was filtered, and the resulting cake was washed with water (3 l) and subsequently extracted with MeOH (3 l). The supernatant and the wash water were subjected to a Diaion HP-20 resin column eluting with 95% EtOH (5 l). The MeOH extract and the EtOH eluents were evaporated under reduced pressure to 1 l at 50 °C, and the resulting concentrate was extracted three times using an equal volume of EtOAc. The combined EtOAc phase was concentrated under reduced pressure to yield a mixture (23 g). The mixture was chromatographed on a silica gel (Qingdao Haiyang Chemical Group, Qingdao, Shandong, China; 100–200 mesh) column and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (100:0–50:50, v/v) to obtain three fractions Fr.1–Fr.3 based on the TLC profiles. TLC was performed on silica-gel plates ($HSGF_{254}$, Yantai Chemical Industry Research Institute, Yantai, China), with solvent system of $CHCl_3$/MeOH (9:1). The developed TLC plates were observed under a UV lamp at 254 nm or by heating after spraying with sulfuric acid/ethanol, 5:95 (v/v). The Fr.2 was subjected to a Sephadex LH-20 gel (GE Healthcare, Glies, UK) column eluted with $CHCl_3$/MeOH (1:1, v/v) and detected using TLC to give two fractions (Fr.2-1 to Fr.2-2). The Fr.2-1 was further isolated with semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μM, 250 × 9.4 mm inner diameter; 1.5 ml min^{-1}; 220 nm; Agilent, Palo Alto, CA, USA) eluting with CH_3CN/0.1% formic acid (30:70, v/v) to give compound 1 (t_R 8.2 min, 83 mg). ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany). The electrospray ionization mass spectrometry (ESIMS) and high resolution

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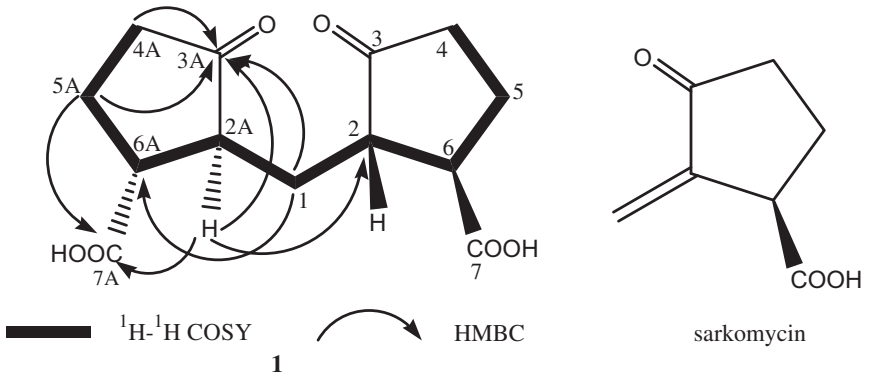

Figure 1 Structures of **1** and sarkomycin, and the key ^{1}H-^{1}H COSY and HMBC correlations of **1**.

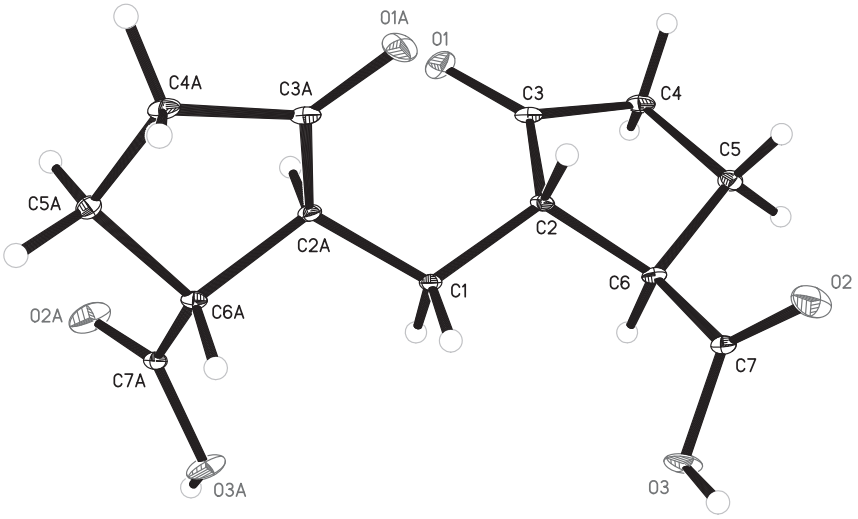

Figure 2 The X-ray crystallographic structure for **1**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 1 ^{1}H and ^{13}C NMR data[a] for compound 1

Position	δ_H	δ_C
1	1.69t (6.8)	27.7 (t)
2, 2A	2.56m	48.6 (d)
3, 3A		217.3 (s)
4, 4A	2.24m	36.3 (t)
5, 5A	1.85m	24.2 (t)
	2.19m	
6, 6A	2.77m	46.4 (d)
7, 7A (COOH)	12.4 broad s	175.3 (s)

[a]Chemical shifts are reported in parts per million (δ), using DMSO-d_6 (δ_H 2.50 p.p.m.; δ_C 39.5 p.p.m.) as an internal standard, with coupling constants (J) in Hz.

electrospray ionization mass spectroscopy (HRESIMS) spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters Co, Milford, MA, USA).

Compound **1** was isolated as a colorless crystal (in MeOH) with m.p. 237–239 °C, $[\alpha]_D^{25}$ + 19.4(c 0.2, EtOH) and UV (EtOH) λ_{max} nm (log ε): 284 (2.12). Its molecular formula $C_{13}H_{16}O_6$ with 6 °C of unsaturation was determined using HRESIMS at *m/z* 269.1015 $[M+H]^+$ (calculated as 269.1020 for $C_{13}H_{17}O_6$). The IR spectrum displayed absorption band for carbonyl (at 1725 cm^{-1}) functionality. Analysis of the ^{1}H and ^{13}C NMR data (Table 1) with the aid of DEPT experiment revealed the presence of three methylenes (δ_C 24.2, 27.7, 36.3), two aliphatic methines (δ_C 46.4, 48.6), one carboxyl carbonyl carbon (δ_C 175.3) and one carbonyl group (δ_C 217.3). The ^{1}H-^{1}H COSY correlations of H-2A/H-6A/H-5A/H-4A (Figure 1) established the C-2A-C-4A structural fragment. The observed HMBC correlation networks of H-2A, H_2-5A and H_2-4A/C-3A; H-2A, H-6A and H_2-5A/C-7A revealed the presence of a 3-oxocyclopentanecarboxylic acid moiety. The methylene group at δ_C 27.7 was connected to C-2A by the ^{1}H-^{1}H COSY correlation of H_2-1 and H-2A and the HMBC correlations from H_2-1 to C-3A and C-6A. Taking the molecular formula and ^{13}C NMR of **1** into account, **1** was probably included two 3-oxocyclopentanecarboxylic acid substructures. The linkage of the two 3-oxocyclopentanecarboxylic acid moieties via C-1 was corroborated by the HMBC correlations between H-2A/C-2 and H-2/C-2A. To secure the assigned structure and also to determine its absolute configuration, a suitable crystal of **1** obtained from MeOH was subjected to X-ray diffraction analysis (Figure 2). The X-ray result not only completely agreed with the proposed structure but also

allowed unambiguous assignment of its absolute configuration with absolute structure parameter 0.10(13) based on the final refinement on the Cu Kα data. Thus, the structure of **1**, including the absolute configuration, was confidently assigned.

Crystal data of compound **1** were collected using a Bruker APEX-II CCD with a graphite monochromated Cu Kα radiation, $\lambda = 1.54184$ Å at 140(2) K. Crystal data: $C_{13}H_{16}O_6$, $M = 268.26$, monoclinic, space group C2; unit cell dimensions were determined to be $a = 18.7920(4)$ Å, $b = 5.82020(10)$ Å, $c = 5.56990(10)$ Å, $\alpha = 90°$, $\beta = 103.1570(10)°$, $\gamma = 90°$, $V = 593.21(2)$ Å^3, $Z = 2$, $D_x = 1.502$ Mg m^{-3}, F (000) = 284, μ (Cu Kα) = 1.013 mm^{-1}. Overall, 2485 reflections were collected until $\theta_{max} = 69.346°$, in which independent unique 992 reflections were observed [R(int) = 0.0421]. The structure was solved by direct methods using the SHELXS-2013 program (Sheldrick, 2013), and refined by the SHELXL-2013 program and full-matrix least-squares calculations. In the structure refinements, non-hydrogen atoms were placed on the geometrically ideal positions using the 'ride on' method. Hydrogen atoms bonded to oxygen were located by the structure factors with isotropic temperature factors. The final refinement gave $R = 0.0434$, weighted residual factors (RW) = 0.1119, Flack = 0.10(13). Crystal data of **1** were deposited in the Cambridge Crystallographic Data Centre (CCDC 1054654).

The cytotoxicity of **1** was assayed for growth-inhibition activity *in vitro* against human cervical carcinoma HeLa cells using the sulforhodamine B (SRB) method.[11] As a result, **1** showed poor cytotoxicity against the HeLa cells (7.3% inhibition at the dose of 100 μM), whereas the IC_{50} value of the positive control (doxorubicin) was 0.234 ± 0.015 μM.

The antimicrobial activity of **1** was assessed against the pathogenic bacterium *Staphylococcus epidermidis* with the broth microdilution MIC method recommended by the Clinical and Laboratory Standards Institute Standards[12] using kanamycin monosulfate as a positive control. Compound **1** showed weak antibacterial activity (MICs: 1, 10 mg ml^{-1}; kanamycin monosulfate, 0.08 mg ml^{-1}).

In previous report, sarkomycin exhibited strong cytotoxicity against HeLa cells.[5] In this research, the bioassay showed that **1** exerted unfavorable cytotoxic activity on this cell line. This may be due to the reduction of the α, β-unsaturated ketone moiety in **1**. This structural subunit existing in many natural products was demonstrated to be the key factor responsible for their antitumor activities and it was weak or inactive when the α, β-unsaturated ketone moiety was absent.[13] Studies on the biosynthetic pathway and other bioactivities of **1** are currently underway.

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